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VARIATION IN TOTAL LENGTH OF FRESH AND PRESERVED BROWN SHRIMP (*PENAEUS AZTECUS* IVES) MEASURED BY TWO METHODS[1]

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ABSTRACT

Brown shrimp (*Penaeus aztecus*), measured by placing the rostrum over the zero mark on a measuring scale, showed no detectable changes in total length after being preserved in 10% buffered formalin for 16 weeks. An apparent decrease in length occurred when the preserved shrimp were measured by placing the rostrum against a block on a measuring scale. This apparent decrease was attributed to the flexibility of the rostrum of the preserved shrimp. Between-observer variation in measurements of mean length was greater than within-observer variation. Differences in estimates of mean length obtained by four observers ranged between 1.5 and 3.8% and were not improved by changing the method of measurement.

INTRODUCTION

Length measurements of shrimp are often made on preserved specimens. It is necessary, therefore, to know if the lengths change after preservation. Also, in-

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formation is needed on the variability in length measurements among different observers as well as in those measurements obtained by different methods.

Lindner and Anderson (1956) measured 300 live white shrimp (*Penaeus setiferus*) and remeasured them after they had been preserved in 10% formalin for 1 day, 1 week and 3 weeks. The shrimp ranged from 96 to 170 mm total length (tip of rostrum to tip of telson). Differences in mean lengths of shrimp measured at different times were less than 1% and were attributed to measurement error.

The study reported herein was designed to determine if the information on the effects of preservation developed by Lindner and Anderson (1956) for white shrimp 96 to 170 mm total length was applicable to brown shrimp 25 to 99 mm total length. Additional objectives were to define the variation involved when different investigators measure shrimp, to evaluate two methods used for measuring shrimp and define the variation associated with each method.

EFFECTS OF FORMALIN

Six hundred small brown shrimp, collected with an otter trawl from Galveston Bay, Texas, were kept on ice until their total lengths were measured within 24 hours after capture. They were then preserved in 10% buffered formalin.

The shrimp were divided into three groups of 200 each according to length: 25 to 49, 50 to 74 and 55 to 99 mm. Each length group was then subdivided into five samples of 40 shrimp each and each sample was preserved for 1, 2, 4, 8 or 16 weeks. The same person measured the fresh and preserved shrimp by placing the rostral tip directly over the zero mark on the scale rather than letting the tip of the rostrum come in contact with a block fixed to the measuring board at the zero mark.

Differences in mean length of fresh and preserved shrimp were negligible. The greatest difference was 1.3% (Table 1). We concluded that the length did not change in juvenile brown shrimp preserved as long as 16 weeks in 10% buffered formalin.

TABLE 1

Average lengths (mm, tip of rostrum to tip of telson) before and after preservation in 10% formalin for different periods and percentage differences for small brown shrimp of three length groups

Period of preservation (weeks)	Length group (mm)								
	25–49			50–74			75–99		
	Fresh	Preserved	Difference (percentage)	Fresh	Preserved	Difference (percentage)	Fresh	Preserved	Difference (percentage)
1	34.47	34.62	0.44	54.20	54.07	—0.24	80.42	80.05	—0.46
2	35.52	35.12	—1.13	53.82	53.70	—0.22	81.15	81.15	0.00
4	43.10	43.45	0.81	53.25	53.40	0.28	81.80	82.47	0.82
8	42.57	42.50	—0.16	57.62	56.87	—1.30	88.25	88.32	0.08
16	35.97	36.00	0.08	61.50	62.30	1.30	88.15	87.88	—0.31

VARIATION BETWEEN OBSERVERS IN RELATION TO MEASURING TECHNIQUE

Two samples of 40 shrimp each were used to determine the difference in measurements of mean length by four trained observers. All shrimp in each sample were measured while fresh and after 1 week in formalin by placing the rostral tip against a block at the zero mark on the scale.

Differences in mean length of samples of shrimp were greater between observers when determined from preserved samples than from fresh samples (Table 2). The observers noted that they had difficulty in measuring the preserved shrimp accurately because the rostrum had become flexible during preservation and bent easily when placed against the block on the measuring scale. The mean length measured by each observer was shorter after the shrimp had been preserved than when they were fresh (Table 2).

Because of apparent bias caused by the flexible rostrum on preserved shrimp, two additional samples of 40 shrimp each were measured fresh and after 1 week in formalin by the same four observers by placing the rostral tip over the zero mark on a scale. This method apparently eliminated the bias (Table 3); the number of overestimates in mean length were equal to the number of underestimates, using mean length estimates of fresh shrimp samples as the base. The difference in estimates of mean length between observers ranged from 1.6 to 2.9%, whereas the greatest within-observer variation was about 1.2% (Table 3).

In conclusion, (1) differences between mean lengths of fresh and preserved brown shrimp 25 to 95 mm total length were negligible, (2) placing the rostral tip of the shrimp over the zero mark of the scale rather than against a block on the zero mark was an unbiased and rapid technique for measuring shrimp and (3) between-observer variation was greater than within-observer variation regardless of the method of measurement.

TABLE 2

Average lengths (mm, tip of rostrum to tip of telson) of 40 small brown shrimp in each of two samples, before and after preservation for 1 week in 10% formalin, as measured by four observers by placing the rostral tip against a block

	Sample 1			Sample 2		
Observer	Fresh	Preserved	Difference (percentage)	Fresh	Preserved	Difference (percentage)
A	73.47	73.42	—0.07	63.02	62.95	—0.11
B	74.22	73.00	—1.64	63.82	62.85	—1.52
C	74.42	73.05	—1.84	63.97	61.20	—4.33
D	74.57	74.25	—0.43	64.00	63.53	—0.73
Maximum difference (percentage) in mean length determined by different observers	1.50	1.71		1.56	3.81	

TABLE 3

Average lengths (mm, tip of rostrum to tip of telson) of 40 small brown shrimp in each of two samples, before and after preservation for 1 week in 10% formalin, as measured by four observers by placing the rostral tip over the zero mark

	Sample 1			Sample 2		
Observer	Fresh	Preserved	Difference (percentage)	Fresh	Preserved	Difference (percentage)
A	107.70	108.13	0.40	81.54	81.20	—0.42
B	109.57	109.73	0.15	81.67	82.40	0.89
C	107.65	106.60	—0.98	80.55	81.11	0.70
D	110.10	108.83	—1.15	82.21	81.98	—0.28
Maximum difference (percentage) in mean length determined by different observers	2.28	2.94		2.06	1.59	

LITERATURE CITED

Lindner, M. J. and W. W. Anderson. 1956. Growth, migrations, spawning and size distribution of shrimp, *Penaeus setiferus*. Fishery Bull. Fish Wildl. Serv. U.S. 56: 553–645.

THE TAPETUM LUCIDUM OF THE STURGEON

J. A. C. Nicol

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ABSTRACT

The chorioidal tapetum lucidum of the sturgeon (*Acipenser transmontanus*) is made up of oriented reflecting cells containing thin flat crystals. Reflecting planes of the cells and contained crystals are tangential to the surface of the retina in the central fundus; towards the periphery they become increasingly oblique. Because of this arrangement cells lie in planes generally normal to incident light. Long outer segments of the rods penetrate deeply into the pigment epithelium and conduct light to the tapetum. Some consequences of these arrangements are discussed.

INTRODUCTION

The eye of the sturgeon contains a chorioidal tapetum lucidum. It is organized somewhat like that of elasmobranchs (Gilbert 1961) and its reflecting material seemingly is guanine. Descriptions of the eye are available in Walls (1942) and Rochon-Duvigneaud (1958). The retina contains rods and single cones. A cellular tapetum lucidum lies immediately outside the choriocapillaris, and the chorioid externally is covered by a stratum argenteum. There is some pigment in the pigment epithelium, the presence of which presents certain problems concerning the function of the tapetum lucidum. The present study is largely a histological one in which the organization of the tapetum lucidum has been resolved and consideration paid to the role of the pigment epithelium.

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MATERIALS AND METHODS

White sturgeon, *Acipenser transmontanus* Richardson, were used. They were obtained from shallow fish ponds at Bonneville Dam, Orgeon, where they had been held captive for long periods.

Eyes were fixed in Witmaack's and Helly's fluids (the latter was more satisfactory) and they were embedded in nitrocellulose. Mostly, sections were cut vertically and horizontally at 20 μm; some small pieces of retina were sectioned transversely to secure very thin sections and others were sectioned parallel to the surface to give tangential sections.

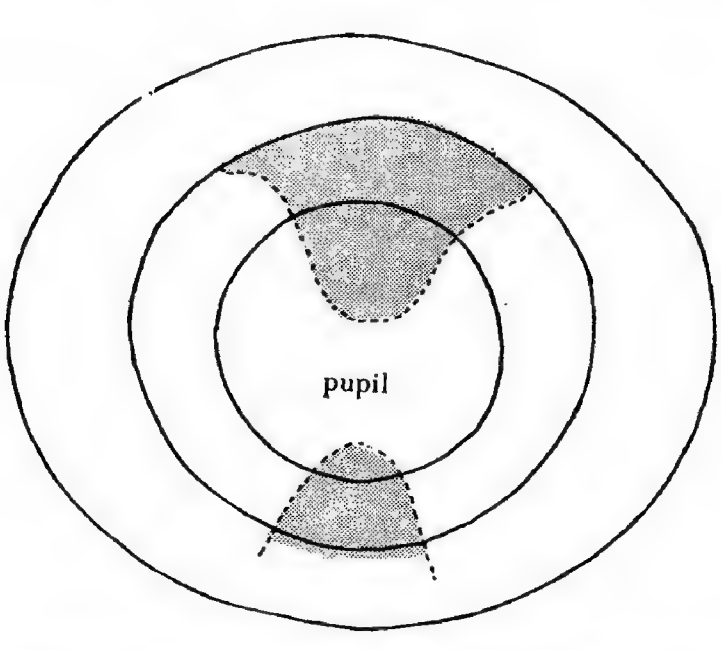
Fresh tissues, immersed in teleost Ringer, were examined with a vertical illuminator, using a Leitz No. 3 objective.

OBSERVATIONS

Examination of Fresh Material

The eyeball is somewhat movable and it is surrounded by an eyelid. The pupil is circular and fixed. The cornea outside is rather cloudy; a triangular patch of black pigment below, sometimes above and below, intrudes over the pupil (Fig. 1). A cornea was gently scraped, outside and inside, without removing the dark pigment; it was concluded that the pigment extends through the stroma of the cornea. These fish were very dark and the skin was heavily charged with black pigment. Mr. Ivan J. Donaldson, Fisheries Research Biologist at Bonneville Dam, reports that they darkened greatly after capture; possibly, corneal pigment increased at the same time.

The eye exhibits subdued eyeshine from most angles, that is to say when it is viewed from below and either side; but the pupil is dark when it is viewed from above. When an eye is removed from the head and cleaned of its fibrous coat a



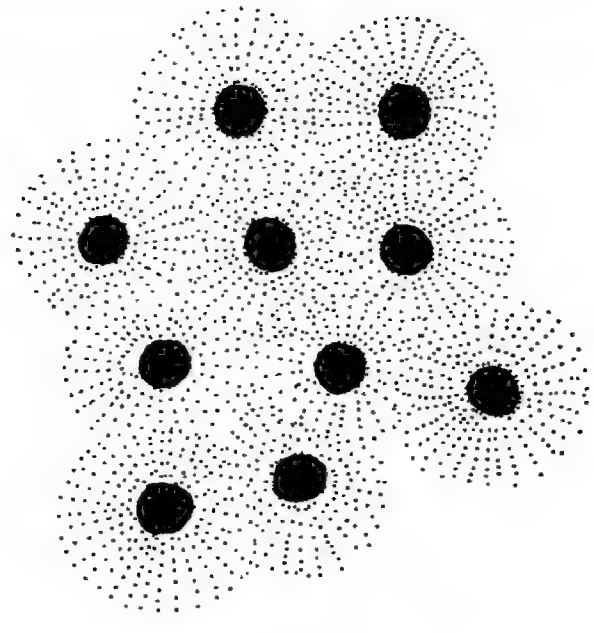


FIG. 1. Appearance of the eye of the sturgeon, viewed from the front. × 8 ca.

FIG. 2. Free-hand sketch of the appearance of the surface of a fresh preparation of the wall of the eye, from which the retina had been removed. Central fundus. Pigmented pegs of the pigment epithelium cells are spaced fairly regularly over the tapetal surface. × 285 ca.

golden tint shows through the translucent sclera. Upon opening a light-adapted eye the fundus appears light yellow, except for the ventral field. An area extending from the ventral margin of the eye towards the central fundus is dark; its upper margin is convex. A black streak, apparently the embryonic fissure, runs from the ventral margin of the eye dorsally towards the center. When the retina is lifted off, the underlying chorioid appears pale gold. The chorioid is brightly reflecting both on its inner and its outer surfaces.

When an eye from which the retina has been removed is examined microscopically, the tapetum lucidum presents a speckled appearance; the surface showing many small black spots which belong to the pigment epithelium (Fig. 2). These are fairly regular in size and distribution, being about 15 μm in diameter and lying 20 to 75 μm apart. Towards the ventral tapetum the spots become larger and the intervals between them less; in the region of the dark ventral field the spots are large, about 30 μm in diameter and the intervals between them only some 7 to 15 μm.

The reflecting surface of the tapetum lucidum shows through between the black spots in the form of a light meshwork. Much of the surface is dull or diffusely reflecting, possibly because of the pigment epithelium; when the latter comes away with the retina or when it is gently scraped off, the bright tapetum lucidum is revealed. The surface of the latter, overall cream-colored, shows minute colored areas of blue, yellow and red; *i.e.*, it is a mosaic of minute multicolored areas. Minute crystals are released from damaged areas, the crystals are very thin hexagonal plates (Fig. 11) resembling those of the tapetum lucidum of a selachian. They have a great size range, 1.9 to 10 μm wide and 3.2 to 28 μm long. Generally the length is 2× or 3× the width. Attempts to determine the orientation of the reflecting platelets were unsuccessful. Mounted on a gimbals table and examined by vertical illumination, the surface of the tapetum reflected light to the greatest extent over an arc of about ± 12°.

Sturgeon, dark-adapted or killed at dusk, had rose-colored retinae. Tapeta lucida of dark-adapted eyes had an appearance like those of light-adapted eyes. It seems, therefore, that no occlusion of the tapetum lucidum takes place.

Histology

Cornea. The dorsal and ventral cornea above and below the pupillary border contains dark pigment cells which have long processes. The cells and their processes lie between the lamellae of the stroma and extend between the epithelial cells. The pigment is particularly dense in the stroma and it extends a short distance over the pupil; the central pupillary area is largely free of pigment. These observations on the distribution of pigment agree with those made on fresh eyes.

Chorioid and tapetum lucidum. The tapetum lucidum contains long narrow reflecting cells which are closely packed (Figs. 3 and 4). They are about 20–30 μm long and 2 μm wide (in section). The nucleus is narrow, elongate and located about midway along the cell. The cytoplasm is loaded with absorbing material appearing as fine striae, superposed and aligned along the length of the

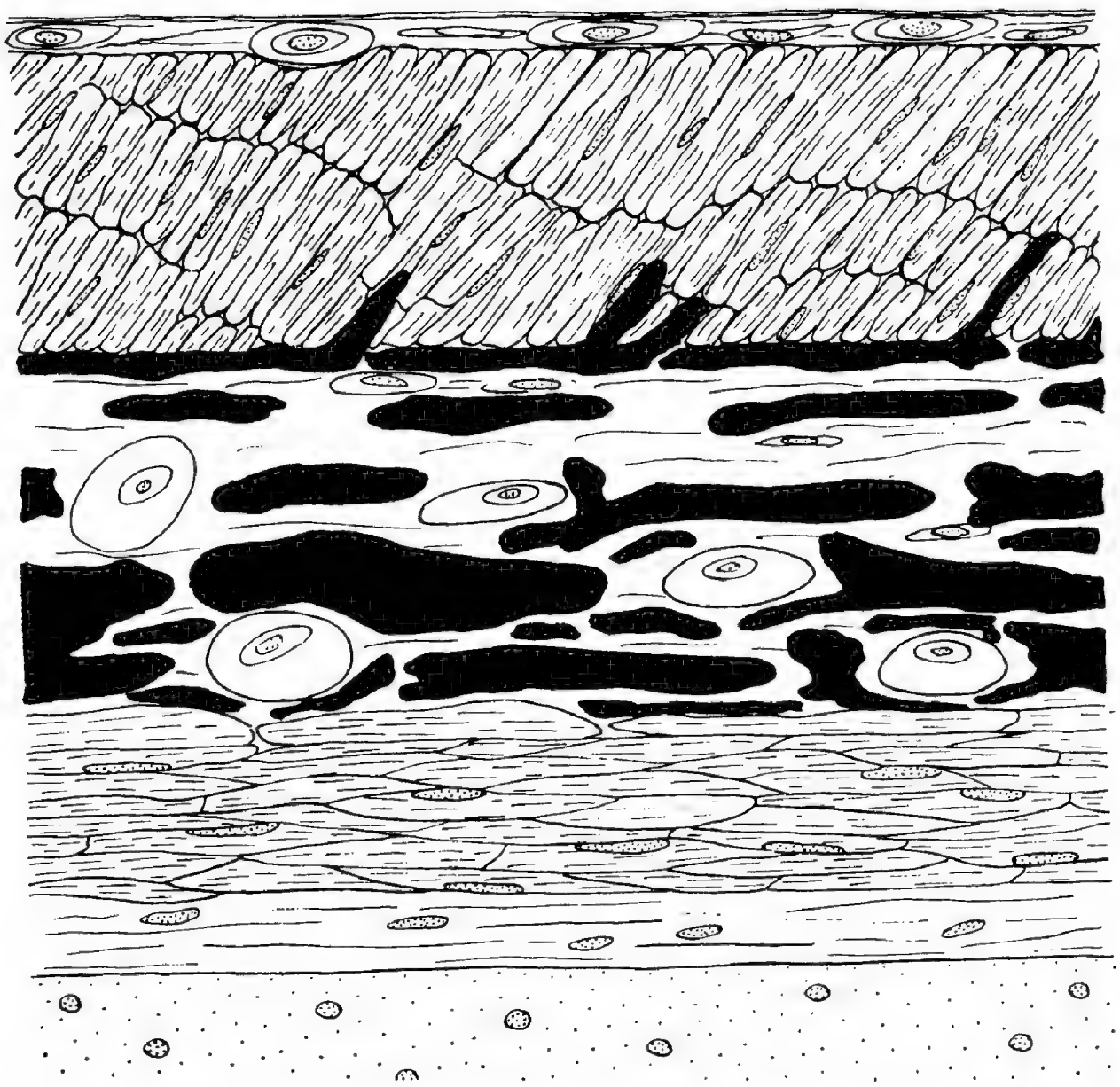


FIG. 3. Semidiagrammatic representation of a section through the chorioid of a sturgeon. The section lies towards the periphery of the eye. × 1,000. Legend: b.v., blood vessel. ch.c., choriocapillaris. el.c., ellipsoid of cone. el.r., ellipsoid of rod. o.n.l., outer nuclear layer. o.s.c., outer segment of cone. o.s.r., outer segment of rod. p.c., pigment cells of chorioid. p.e., pigment epithelium. p.p., pigmented pegs. rf.c., reflecting cells of tapetum lucidum. s.ch., suprachorioid. st. arg., stratum argenteum.

cell parallel to the surface. The cells are very similar to those of elasmobranchs (Best and Nicol 1967). Like the latter, the cells probably contain thin, flat crystals arranged as piles of lamellae (the platelets), which are visible optically and as striae histologically.

In the central fundus the reflecting cells lie parallel to the lamina vitrea and

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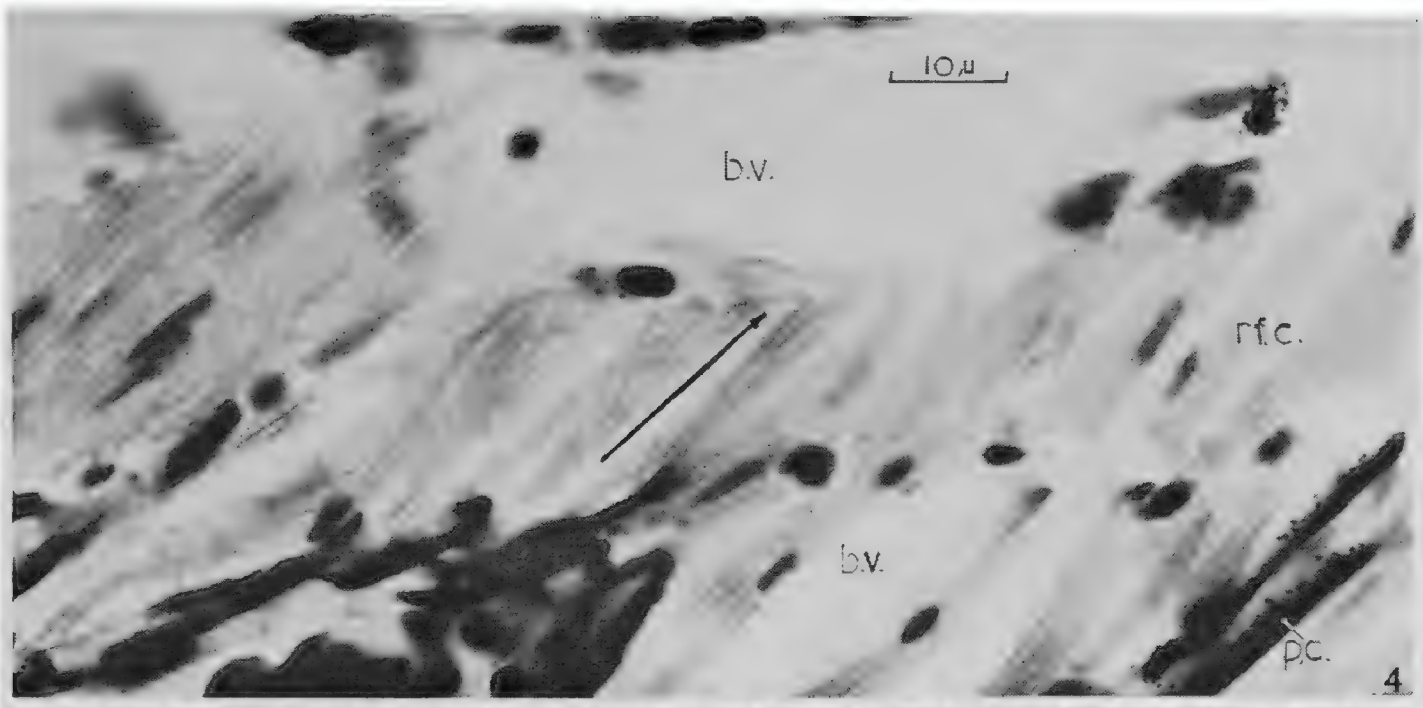
FIG. 4. Transverse section through the ventral tapetum lucidum. Arrow shows orientation of reflecting cells and platelets.

FIG. 5. Transverse section through the ventral retina and chorioid.

FIG. 6. Tangential section through outer retina, level of pigmented pegs (p.p.).

FIG. 7. Slightly oblique tangential section through pigment epithelial cells. At left, the basal region of the pigmented peg is in evidence; at right the section passes below the pigmented region, and outer segments (of rods) lie well within the basal nuclear region of the pigment epithelial cell. For clarity, some outer segments have been ringed. Scale of Figs. 5, 6 and 7 same as that of Fig. 4.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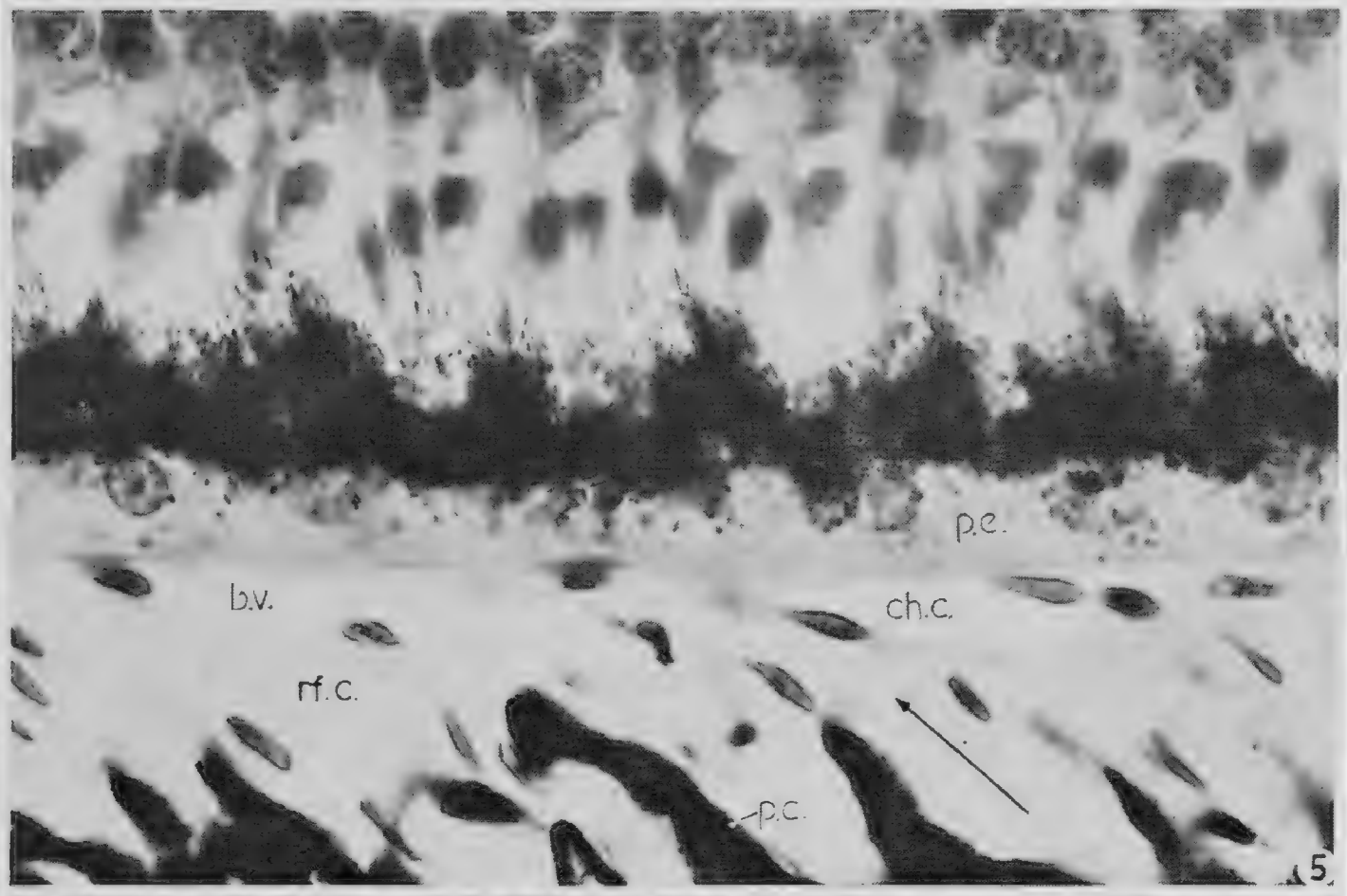
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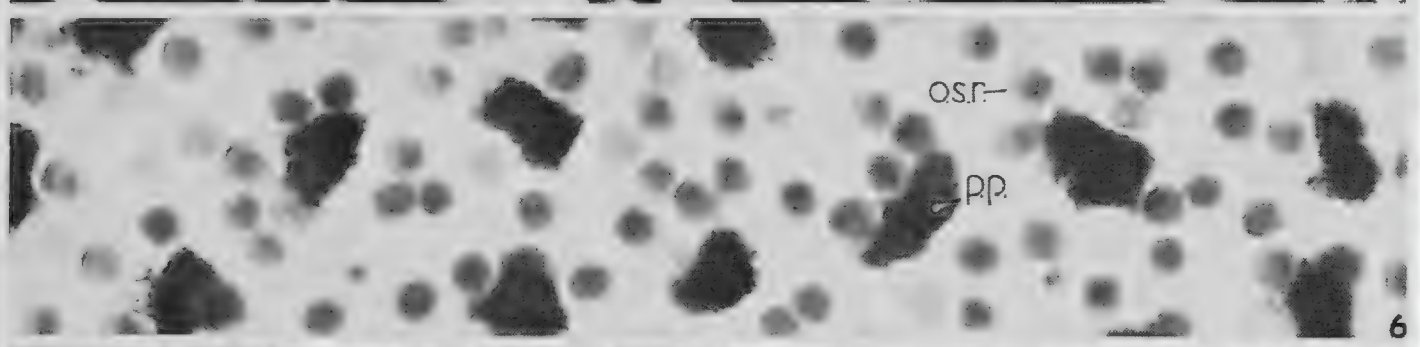
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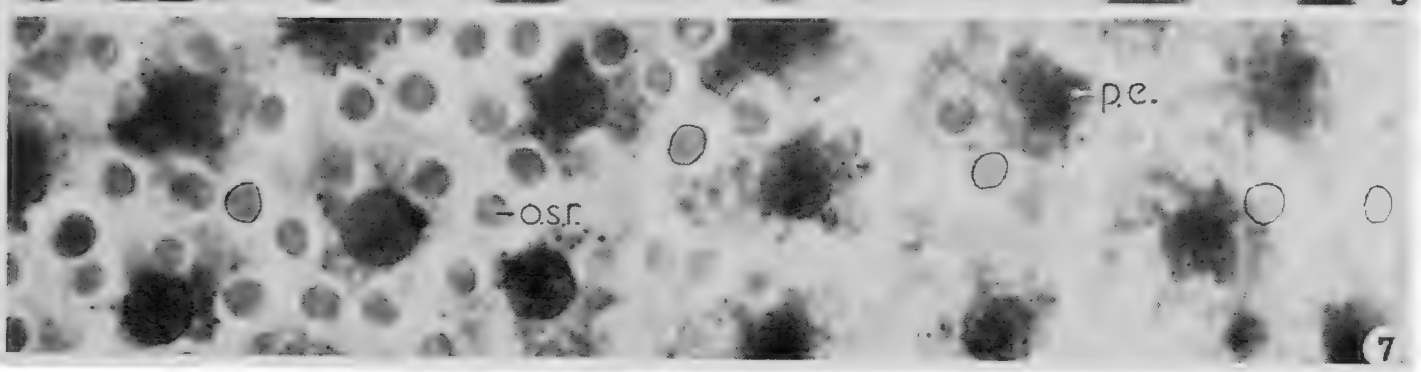
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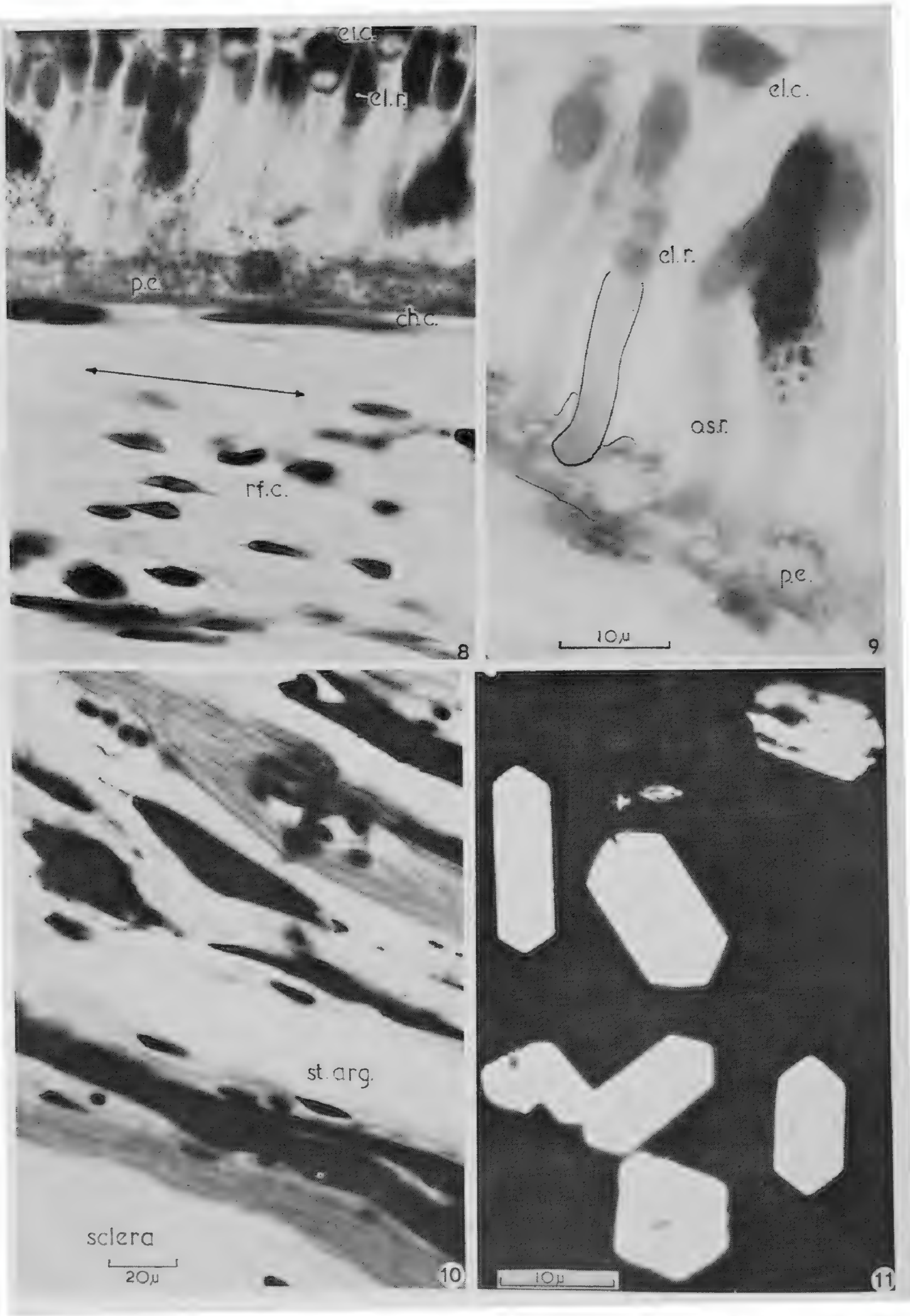


FIG. 8. Transverse section through the central region. Scale of Fig. 8 same as that of Fig. 10.

FIG. 9. Transverse thin section showing rod outer segment penetrating the pigment epithelium (outline of both emphasized by ink).

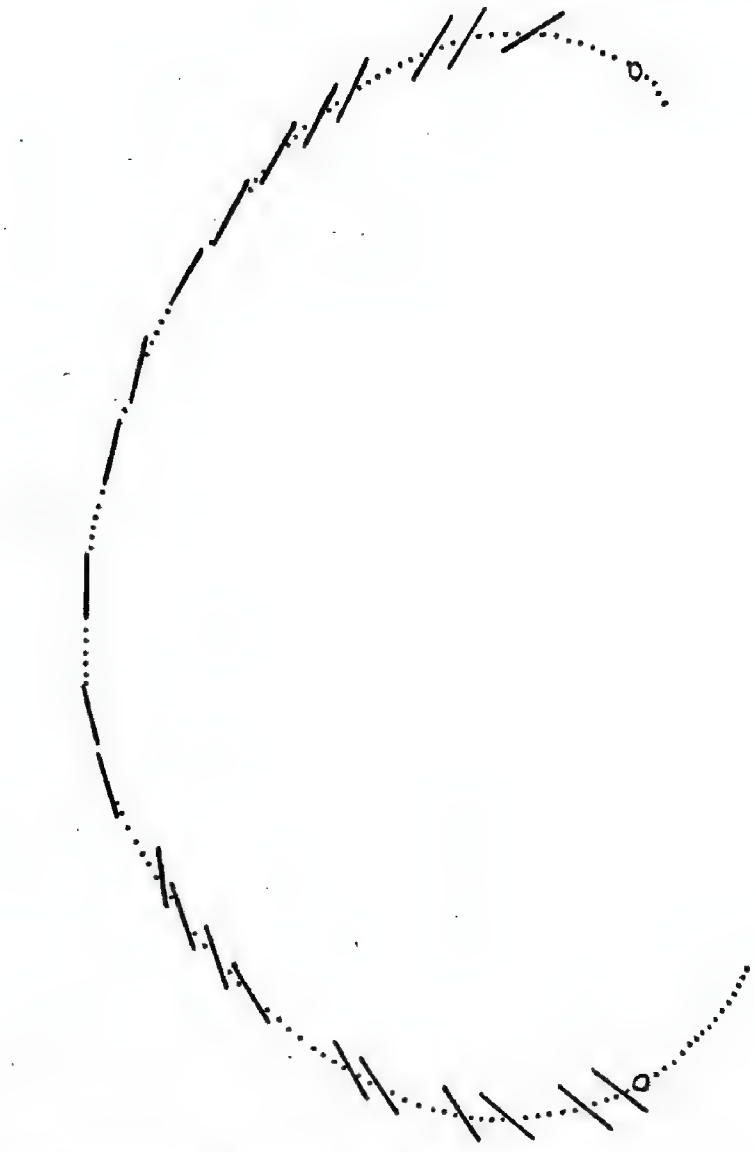


FIG. 12 and 13. Orientations of reflecting cells in vertical and horizontal planes, respectively. Circles (in Fig. 12) indicate limit of ora terminalis.

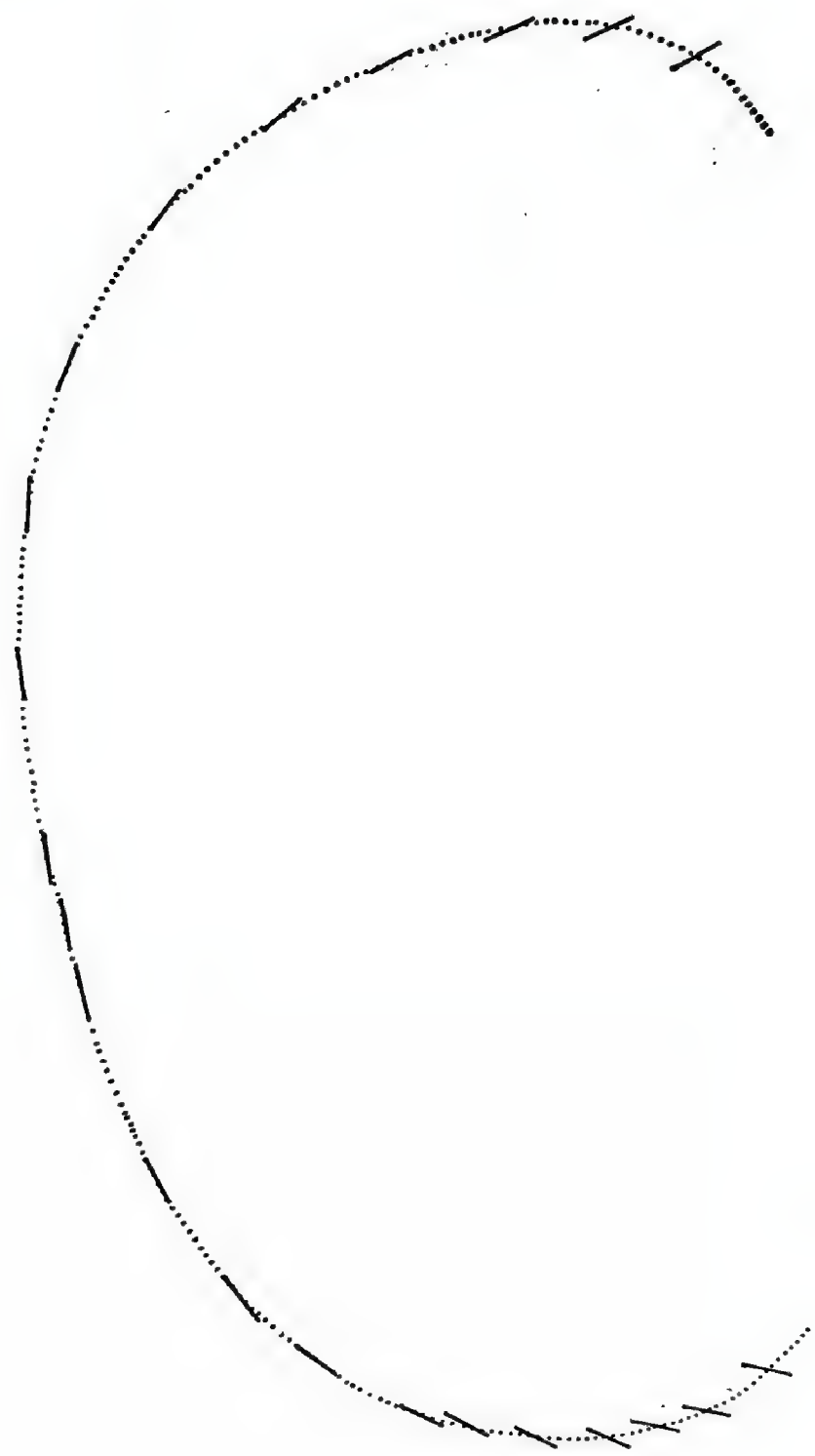
towards the periphery they lie oblique to the latter; obliquity, slight near the center, increases progressively towards the periphery of the eye (Figs. 3–5, 8, 12 and 13).

Centrally, the reflecting cells are layered, about 10 cells deep (Fig. 8). Peripherally, where they are oblique, they are staggered in depth, several cells succeeding each other from the inner to the outer limits of the tapetum (Figs. 3 and 4). Penetrating blood vessels pass through the reflecting layer obliquely (Fig. 4); they are not accompanied by pigmented sheaths as they are in the selachian chorioid (Nicol 1964). Connective tissue fibers staining with aniline blue are present; mostly they are too fine for resolution and give a faint blue tint to the intercellular boundaries.

The outer chorioid is heavily pigmented (Figs. 3 and 10). Towards the periphery of the eye pigmented processes of melanocytes extend short distances inwards between the reflecting cells, but stop far short of the choriocapillaris (Fig. 5). The pigment does not migrate so as to occlude the tapetum lucidum. The stratum

FIG. 10. Transverse section through outer chorioid, at junction with sclera, to show stratum argenteum.

FIG. 11. Crystals from the tapetum lucidum. Vertical illumination.



argenteum of the outer chorioid also contains reflecting cells which lie partly outside the pigmented chorioid, partly within, being partially invested by melanocytes. They lie parallel to the scleral surface (Figs. 3 and 10).

The whole chorioid layer in the central fundus is about 100 μm thick (in an eye having a diameter of 8 mm). The lamina vitrea plus the choriocapillaris is 4–6 μm; the tapetum lucidum is 20 μm, increasing to 30 μm towards the periphery. Over most of the eye the outer chorioid is thin, compact and densely pigmented. In the ventral periphery it becomes much thicker and contains an open meshwork of pigment cells.

Retina. Cones have short peg-like processes whereas rods have long outer segments that extend to the basal cell bodies of the pigment epithelium (Figs. 5–9, 14). Outer segments of rods are about 13 μm long in the dorsal retina, 27 to 29.5 μm in the central and 19 μm (estimated) in the ventral retina. The outer

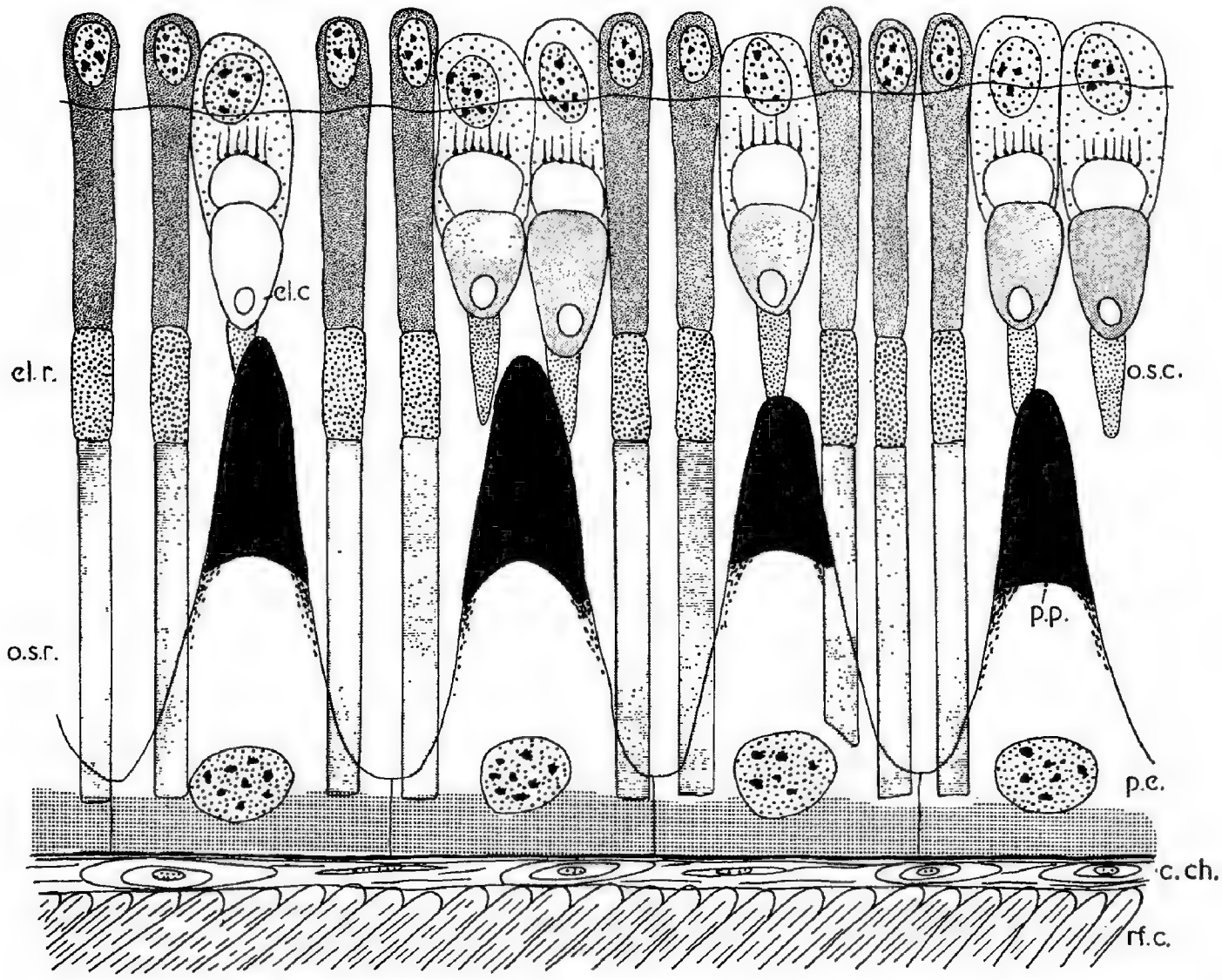


FIG. 14. Drawing of the organization of the outer retina and inner chorioid. Diagrammatic. About 430 ×.

segments of the rods and cones are distributed somewhat irregularly. Rod outer segments are thick, about 4.3 μm in diameter; distances between them are 0.5× to 2× rod thickness. Outer ends press far into the pigment epithelium, in recessed pockets, and lie very close to the tapetum from which they are separated by an interval of about 8 μm (Figs. 7 and 9). They are absent from the region immediately above the nucleus of the pigment cell, but invest it in a close ring (Fig. 7).

Cell bodies of the pigment epithelium are long rectangles (in section) largely free of pigment. From each cell arises a pigmented peg (process) which extends inwards among the receptor elements. Pigmented pegs are about 30 μm long in the central and dorsal fundus and 20 μm long in the ventral fundus. In the central fundus, pegs are around 8–16 μm wide and 12 to 77 μm apart. In general, pegs of two contiguous pigment cells are about 20 μm apart; greater intervals are probably caused by the plane of sectioning missing them (*cf* Fig. 6). The pigment is in the form of fine elongate granules.

In the dorsal and central retina much of the pigment is generally aggregated in a dense black mass towards the inner end of the peg and dispersed granules lie around the periphery of the peg between the tip and base (Fig. 8). Sometimes the pigment is more dispersed throughout the peg or more concentrated at the tip. In the ventral retina the pigment occupies all the process and extends into

the inner and peripheral region of the cell body (Fig. 5); consequently, the pigment forms a nearly continuous covering.

Several animals were killed in daylight, several at dusk and one after dark. No consistent differences were noted in the disposition of the pigment. In the animal killed after dark, the pigment was aggregated in the inner regions of the pegs to a greater extent than in the other animals. In general, the material seems to show a fair amount of individual variation in distribution of pigment, rather than evidence for migration of pigment. Rods and cones occupied the same position in light and dark eyes.

DISCUSSION

The tapetum lucidum of the sturgeon closely resembles that of selachians in several important respects. In both groups—selachians and chondrosteans—the tapetum lucidum is chorioidal, consisting of a thin layer of flat reflecting cells which are precisely aligned with reference to the surface of the retina. In the central fundus the reflecting cells are parallel to the retinal surface, but towards the periphery they become increasingly oblique to that surface. Rod outer segments are perpendicular to the retinal surface; therefore, they are normal to the surfaces of the tapetal reflecting cells only in the central fundus and elsewhere they form acute angles with the reflecting cells as the latter become tilted away from the retinal surface. Other features in which the eyes of the two kinds of animals resemble each other pertain to the size and shape of the crystalline inclusions, appearance of the reflecting platelets in fresh preparations and the alignment and packing of the platelets in the reflecting cells. The tapeta of both eyes exhibit, in surface view, a medley of colors, which can account for the highly unsaturated condition of reflected light that is revealed by measurements of spectrum reflectivity (Denton and Nicol 1965; Best and Nicol 1967).

Some differences between the sturgeon and the selachian eye may be noted. In the sturgeon eye, but not in the selachian eye, reflecting cells are arranged serially in tandem. There is no provision for occlusion of the tapetum by migration of chorioidal pigment in the sturgeon eye, in contrast to the selachian eye which has such a mechanism.

The arrangement of pigment in the cornea over the dorsal pupil and in the ventral retina is such as to reduce eyeshine. These pigmented areas, in conjunction, reduce the amount of light reflected upwards out of the pupil; they serve, therefore, to make the eye inconspicuous when viewed from above against the dark bottom or dim upward welling light.

The cones, the outer segments of which lie far from the tapetum lucidum, probably derive little or no advantage from the reflectivity of the latter. The tapetum lucidum is part of a scotopic mechanism.

The lid and iris act as stops that can restrict light, coming from dorsal and ventral fields, to the periphery of the lens (Fig. 15). The change of orientation of platelets around the fundus is like the situation obtained in the selachian eye and achieves the same result whereby incident light rays are generally normal to the surfaces of the reflecting platelets (Denton and Nicol 1964).

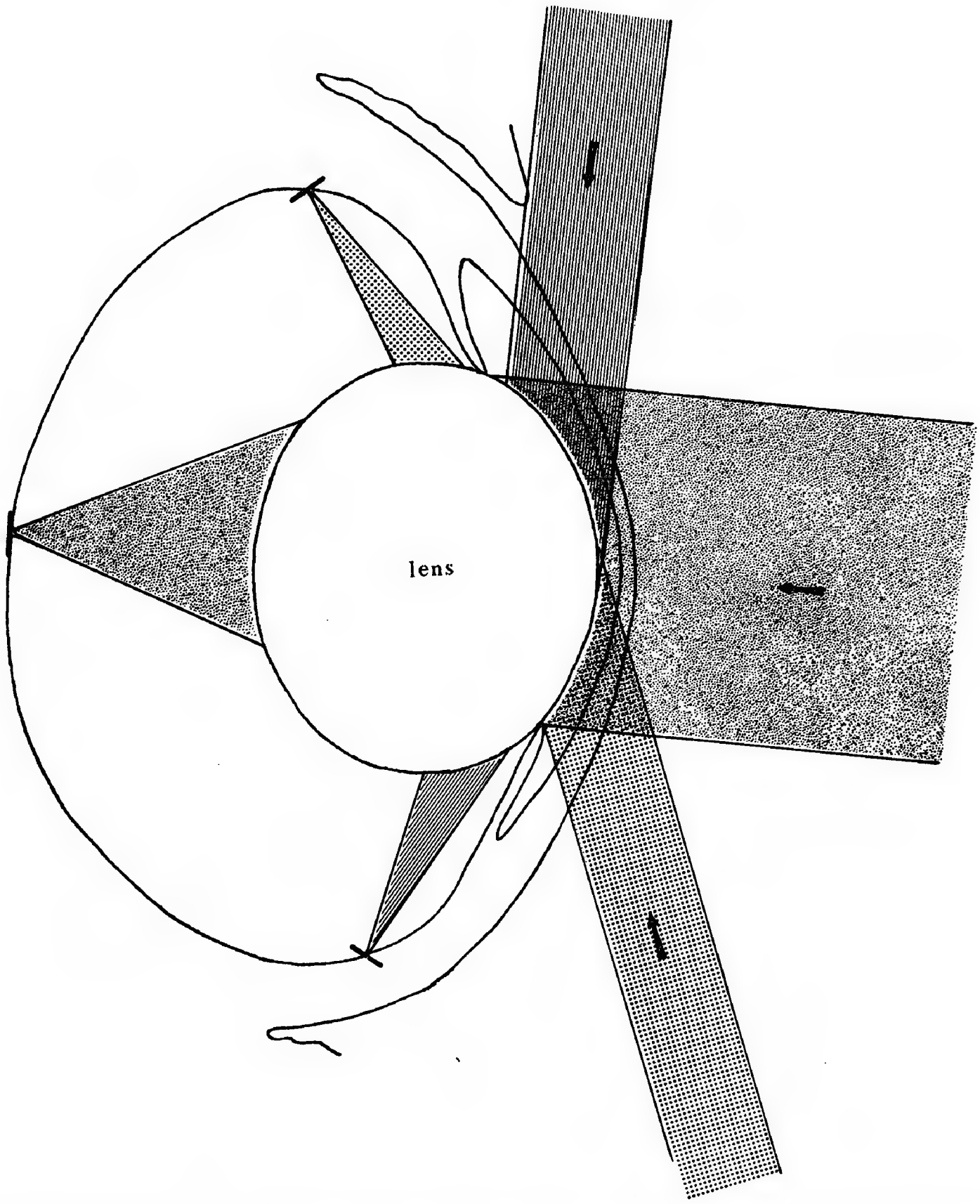


FIG. 15. To show how light-rays, entering three regions of the eye, are oriented with reference to the reflecting platelets.

The outer ends of the rods lie very close to the reflecting cells of the tapetum (the interval is as small as 8 μm); they have an N.A. (in air) of about 0.35 and they are separated from each other by an interval that is often about equal to their diameter. Assuming that the refractive index of the medium is 1.336 and that of the rod outer segment is 1.41 (Sidman 1957), the critical angle for the outer segment is 71°. Some possible consequences of this arrangement in the central fundus are shown in Figure 16, for light rays impinging on tapetal plates tangential to the

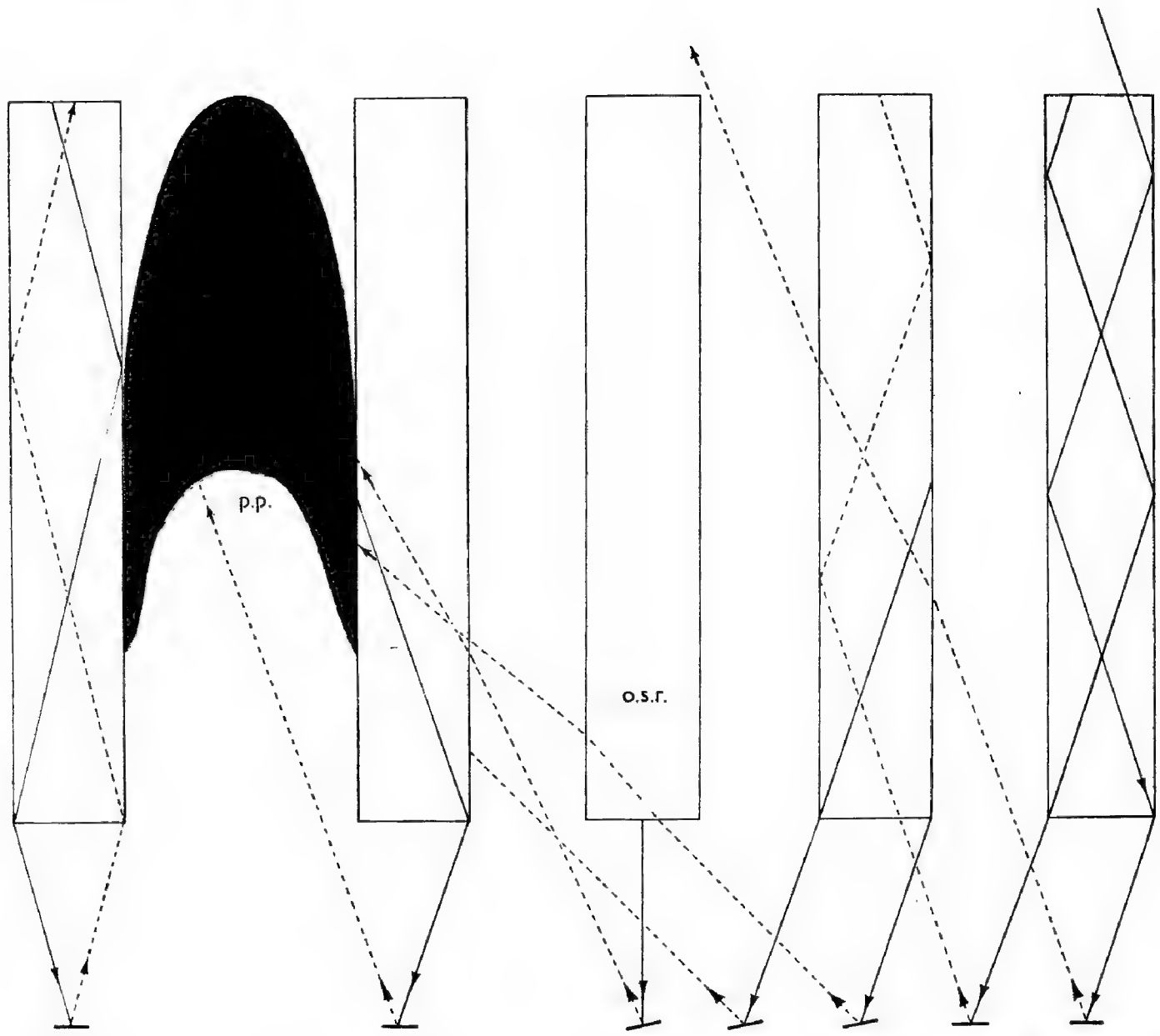


FIG. 16. To show how light, channelled down outer segments of rods, is reflected back into the same or immediately adjacent rods. Reflecting platelets are shown parallel to the tapetal surface, or at 12° thereto. More divergent rays are mopped up by the pigment of the pigment epithelium. To scale.

surface and tilted at 12° to the surface. Rays diverging maximally from the central optic axis are reflected into a neighboring outer segment or they may traverse two neighboring segments. Although the spacing of the rods shows more variation than is indicated in these selected examples, in general light-rays are reflected to a large extent back into the same outer segment which they traversed in first passage or into one or two outer segments near by.

Near the periphery of the eye, where the reflecting cells are tilted to the surface of the retina, the situation is different from that considered above because the rods themselves are not in the axes of the light rays. When the light rays strike the rods at less than the critical angle they pass obliquely through the layer of rods with little change of direction and encounter the reflecting cells nearly normal to their surfaces. Because the platelets are dielectrics, the light reflected from platelets set at considerable angles to the normal may be less effective for vision. The pigmented pegs limit the lateral spread of reflected light. As Walls (1942) indicates, the ratio of ganglion cells to rods being low, there is much convergence

and summation in the system; a factor which may negate the consequences of illumination of several rods by tapetal reflection.

Sturgeons seem to vary a good deal in the degree to which the pigment epithelium is pigmented. The epithelium of *Acipenser ruthenus* lacks pigment except in the anterior region, where processes extending between the visual cells are pigmented. In *A. sturio*, processes everywhere in the retina contain pigment (Rochon-Duvigneaud 1958; Denton and Nicol 1965). There is little pigment in *A. oxyrhynchus* (Ali *et al.* 1968). *A. fulvescens* contains much pigment compactly massed in the tips of the heavy processes (Walls 1942). It is not known what these differences in the distribution of retinal pigment signify. Possibly, in all these species, the rods effectively channel light through the retinal pigment.

A chorioidal tapetum lucidum is a notable feature of the eyes of several groups of fishes which are generally regarded as primitive; *viz.*, elasmobranchs, chimaerids, chondrosteans (sturgeons), cladistians (*Polypterus*) and possibly the Crossopterygii (*Latimeria*) (Walls 1942; Rochon-Duvigneaud 1943, 1958). Whether it should be regarded as a primitive and common feature of these animals or an independent acquisition in each group is an open question, especially since tapeta lucida of the last two groups have been inadequately studied. Certainly, the tapeta lucida of selachians and chimaerids are so much alike that they could well be homologous. The resemblance between the tapeta of selachians and sturgeons is less profound. The ability to utilize oriented reflecting layers containing guanine crystals is very wide-spread among all kinds of gnathostomatous fishes and it is not unlikely that when selective advantage compels, tapeta have appeared as readily as oriented reflecting layers in the dermis. In these various tissues the cells are much the same in their cellular organization and reflecting characteristics, but they differ in their orientations and locations.

ACKNOWLEDGMENTS

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GROWTH, RESPIRATORY METABOLISM AND SEASONAL DISTRIBUTION OF JUVENILE PINFISH (*LAGODON RHOMBOIDES* LINNAEUS) IN REDFISH BAY, TEXAS[1]

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ABSTRACT

The abundance of pinfish on a grass flat area of Redfish Bay, Texas, increases rapidly in late winter, reaches a spring peak and declines through the remainder of the summer and fall to low levels in winter. Pinfish are absent altogether during brief periods in mid-summer and -winter when temperatures exceed lethal levels. Nearby deeper channels provide refuge at these times.

Metabolic rates of the pinfish are effectively temperature compensated. The overall Q_{10} value for the year is about 1.7, but within-season temperature responses are larger. Metabolic depression is evident at temperatures near lethal limits and depression was observed at periods when movements to other more favorable areas occurred.

The period of rapid growth corresponds to times of maximum pinfish density on the grass flats and growth proceeds at a low rate during winter. Comparison of growth rates from several other

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areas shows differences of nearly two-fold in length at the end of the first year. At least some of these differences can be explained on the basis of location and timing of samples from the various areas, as well as size-related movements that occur seasonally.

INTRODUCTION

On the Gulf of Mexico and Atlantic coasts, the pinfish is an abundant member of the fish fauna. It occurs from Cape Cod to Yucatan in a wide variety of habitats; from shallow inshore and bay areas to the open waters of the Gulf and Atlantic ocean. A considerable amount is known about its life history, systematics, distribution and habits (Hildebrand and Schroeder 1928; Hildebrand and Cable 1938; Gunter 1945; Reid 1954; Caldwell 1957). Most of the these studies have been carried out either in shallow, well-vegetated flat areas, or in the more open areas of estuaries and associated channels. In these habitats, the pinfish shows some rather consistent patterns of distribution and abundance, and some substantial differences. The purpose of this study was to investigate the patterns of distribution and abundance on a grass flat in Redfish Bay, Texas and to compare the results with the data already in the literature.

A secondary purpose was to study various ecological and physiological parameters at the same time, in order to obtain at least a partial explanation for the patterns observed. Specifically, changes in the respiratory metabolism, growth and blood were studied; the former two topics will be discussed here and the latter in a later report.

There are now pinfish growth data for several areas: Beaufort, N. C. by Hildebrand and Cable (1938); Cedar Key, Florida by Reid (1954) and Caldwell (1957); Laguna Madre, Texas by Hellier (1962); and Redfish Bay, Texas by Hoese and Jones (1963). Additionally, some very approximate growth rate estimates are provided by Gunter (1945), Kilby (1955) and Hoese (1965). All of these growth data are based on length-frequency analyses of one sort or another and no scale analyses have been reported. Scales have been examined in our laboratory (Wohlschlag and Cameron, unpublished data) but have proved very difficult to interpret. Length-frequency data were necessarily biased by sampling errors, hampered by variability in growth rates within year-classes and, in the case of the pinfish, complicated by patterns of movements that are partially size-dependent. However, the rates probably are reasonable approximations and comparisons of rates from different areas and times should be valid.

Metabolic data also have been published for the pinfish by Wohlschlag and Cameron (1967) and Wohlschlag, Cameron and Cech (1968). In both cases, however, the studies have been concerned with larger fish in different circumstances. Measurements were performed on fasting fish after acclimation in the laboratory for at least 48 hours to constant temperatures. In the present study, measurements were carried out on recently-fed fish under field conditions of temperature and acclimation history.

MATERIALS AND METHODS

Metabolism Calculations

Metabolism is usually expressed as a function of body weight and has the general form:

$$M = aW^{b_w} \tag{1}$$

at any given temperature; where M = total oxygen consumption rate, W = wet weight, and a and b are coefficients. Introduction of temperature into the equation can be accomplished in the following way:

$$M = aW^{b_w} \cdot Q^{t-t_o} \tag{2}$$

where t is the temperature measured about a constant value t_o and Q is the Krogh correction factor approximated for a particular temperature range. Expressing equation (2) in logarithmic form yields:

$$\log M = \log a + b_w \log W + (t-t_o) \log Q, \tag{3}$$

which can be analysed in linear terms. Log a is now replaced by a', and log Q by b_t. Setting t_o equal to zero yields:

$$\log M = a' + b_w \log W + b_t\ (t). \tag{4}$$

Using linear approximations of b_t over short temperature ranges, equation (4) can be fitted by standard regression procedures. This multivariate approach has proved most useful in interpreting metabolic data and is followed in this study.

Description of the Study Area

All fish for field studies were collected from a shallow grass flat adjacent to Hog Island, which lies approximately in the center of Redfish Bay, Texas, at 27° 54′ N, 97° 06′ W (*see* map, Fig. 1). Hog Island is a low remnant of an oyster reef,

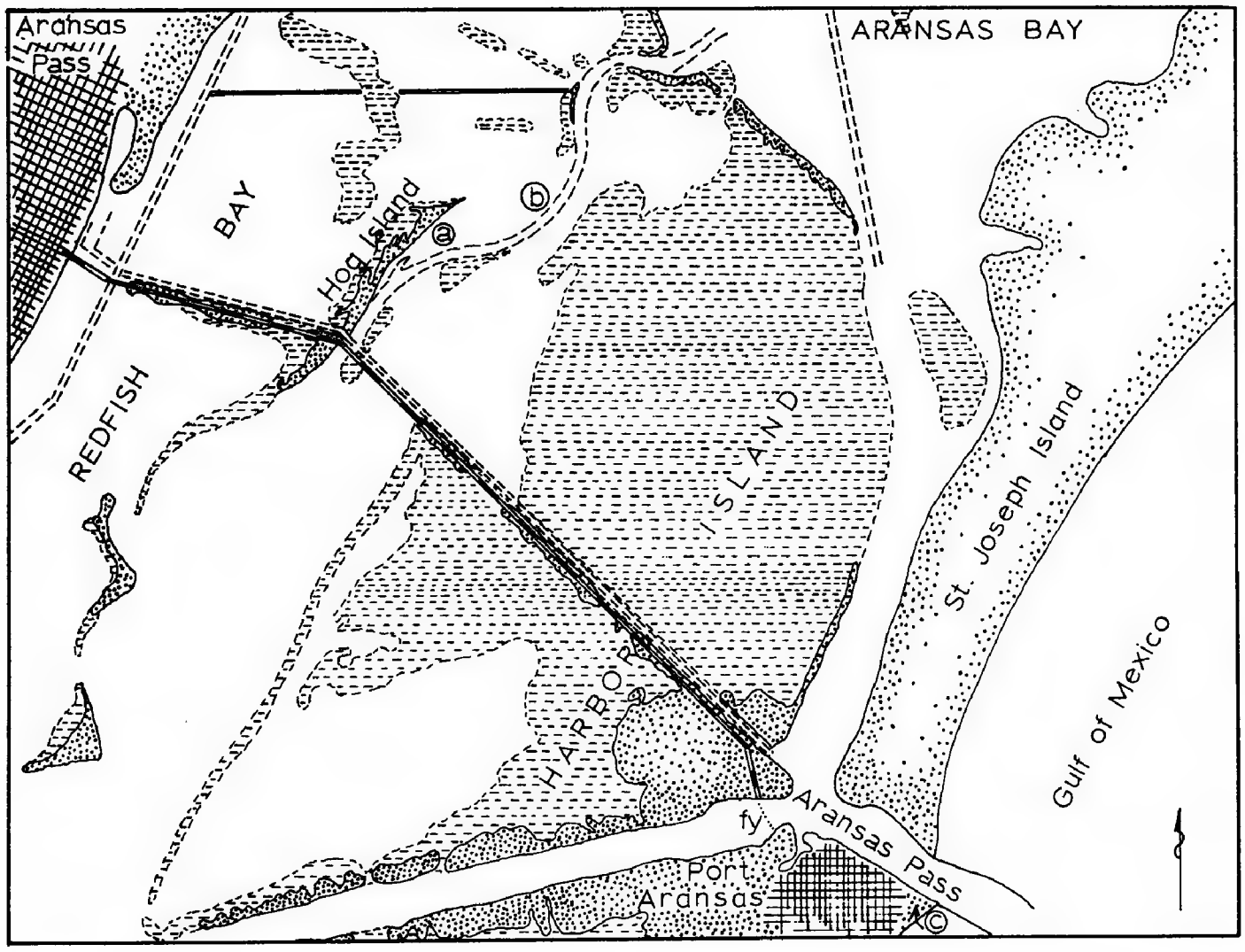

FIG. 1. Map of the study area, showing location of Hog Island in Redfish Bay, Texas and detail of Hog Island Area. a. Hog Island study area. b. Corpus Christi Bayou (dashed lines). c. Marine Science Institute, Univ. of Texas.

which grades gently into a flat area of submerged mud, sand and shell on either side. The bottom is densely vegetated with *Thalassia testudinum* and *Diplanthera wrightii*, commonly called turtle grass and widgeon grass, respectively. Water depths vary with the prevailing winds and tides, but average depth of the study area is about 0.5 m. Tidal influence is small, and is often obscured completely by wind-generated water movements. Strong north winds may lower water levels as much as 0.5 m, exposing many areas of Redfish Bay.

About 300 m distant from Hog Island on the south side, there is a deeper channel, Corpus Christi Bayou (dashed lines, Fig. 1), which formerly provided communication between Aransas Bay on the north and Corpus Christi Bay on the south. Dredged channels to the south, west and east now divert much of the flow from the bayou (Odum 1963). The water depth of the bayou has decreased from over 5 m to about 2 m in the past fifteen years (U. S. C. & G. S. charts and survey by the author). However, there is still sufficient flow from the deeper and more stable reservoirs of the large bays to provide refuge from environmental extremes more common to the flats.

Vegetation, typically sparse in winter, begins to develop in March and April. By early summer dense stands of grasses, which blanket this habitat (Odum *op. cit.*), afford food and cover to a variety of marine organisms, particularly the juvenile stages (Hoese and Jones 1963). Pinfish and other juvenile organisms appear on the flats from winter to mid-summer and persist for varying periods of time according to their life cycles and environmental requirements.

Experimental Procedures

Fish were collected in the vicinity of Hog Island during the period from the summer of 1967 to fall of 1968. For all collections, either a 15- by 2-m bag seine or a 4-m otter trawl was employed. The seine had a mesh size of ¼ inch (6.35 mm) and was fished in approximately the same manner each time so as to sweep an area of approximately 225 m^2. Fish were held in live cars for field work, and in insulated containers for transport to the laboratory.

For some later experiments, fish were caught either by hook and line or in traps adjacent to the Marine Science Institute pier (*see* map, Fig. 1, c). These fish were held at least 24 hours after capture before beginning any experiments.

For measurement of metabolism, freshly caught fish were held in live cars or aquaria for at least one and up to 24 hours after capture, then placed in 2900 ml Fernbach flasks fitted with large stoppers and inflow and outflow tubes. After this initial handling, an hour was allowed for calming and acclimating the fish to their new surroundings.

The oxygen consumption was then measured by the Winkler method for four successive time periods (10 to 30 min), whose length was adjusted for the temperature and the size of the fish. Oxygen concentration was never allowed to fall below 60% of saturation and the oxygen consumption rates showed no oxygen dependence. Blank runs were made each day and an appropriate correction applied.

Successive oxygen content values were then plotted and a least squares regression analysis yielded the rate of consumption (the slope of the regression line).

Variables measured were the rate of oxygen consumption in mg/hr, the weight of the fish in gm and the temperature to the nearest 0.1 C. These values were then tabulated as $\log s_{10}$ for weight and oxygen consumption; in all calculations, Y denotes $\log_{10}$ oxygen consumed in mg/hr, X_w the $\log_{10}$ weight, and X_t the temperature in C. The series of measurements were then grouped according to temperature and season and multiple regression analyses performed according to standard statistical procedures (Steel and Torrie 1960).

In all cases, rates reported should be considered "routine" according to Brett's (1962) terminology.

Growth rate measurements were made by plotting standard lengths of all fish collected in length-frequency histograms. The modes of the frequency groups were calculated and plotted against time. Curves are fitted by inspection. From the anteriormost portion of the mouth parts, with the jaws tightly closed, to the end of the hypural plate was considered to be the standard length.

RESULTS

Distribution and Abundance

The abundance of pinfish on the Hog Island study area from June 1967 to October 1968 is shown in Figure 2. The figures were calculated from numbers of fish caught per seine haul, serially averaged by two's to show trends. The period from mid-December to early January was the only time at which the abundance dropped to zero; other methods of collection were also employed at this time with the same result.

Also shown in Figure 2 are the data for temperature and salinity of the study area at the time of collection. The high values that prevailed in summer of 1967 probably represent a normal high range for this Bay, but the torrential rains and floods that followed Hurricane Beulah (Sept. 20–22, 1967) reduced salinity to 4 ppt two weeks later. The four subsequent drops in salinity (Nov. 1967; Jan., Feb., May 1968) were each due to heavy rains. Each of these periods appears to correspond to a period of reduced abundance of pinfish.

The three periods of lowered salinity in 1968 also correspond to rather sharp decreases in water temperature. The cause of these changes was the cold air fronts that passed over Texas coastal areas at these times. The advent of a "norther" will often change air temperatures as much as 10 C in a few hours and may be attended by winds as high as 40 mph.

During one such period, from December 15, 1967 to January 10, 1968, collections were made with a 4-m otter trawl in Corpus Christi Bayou. Large numbers of pinfish and other species were caught; these fish appeared to be moribund at the time. Temperature of the grass flats, where no pinfish were present, was about 7.6 C; whereas the channel was about 9 C. Many of the species caught in the channel at this time do not normally appear in trawl catches and none had been as easily caught in colder waters.

During another period in August 1968, seine hauls yielded over 100 fish per drag at 32 C and none two days later at 35 C. At night the same day, at about 28 C, large numbers of pinfish were again caught; whereas there had been none earlier in the day.

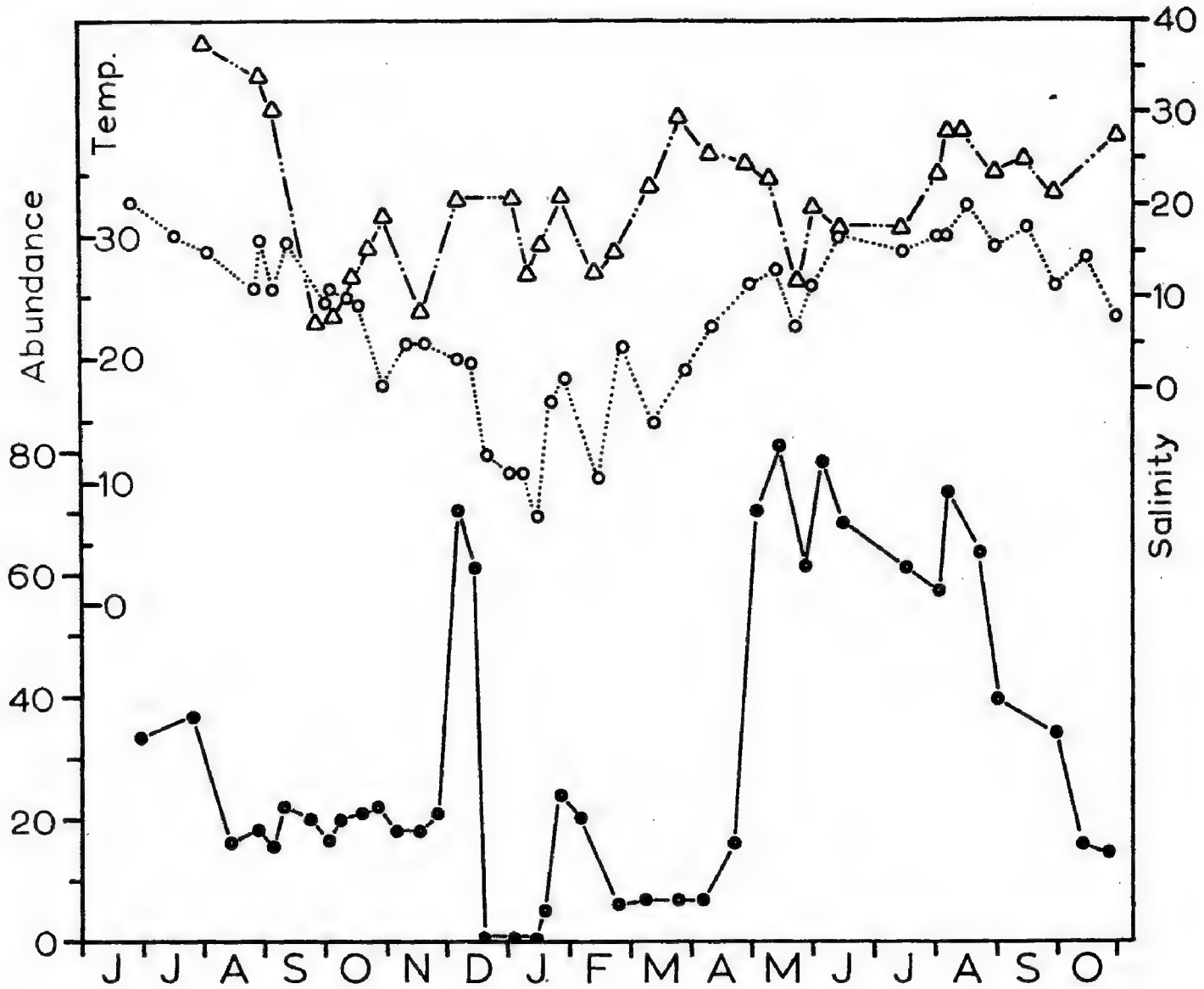

FIG. 2. Temperature, salinity and abundance of pinfish from the Hog Island study area. Temperature (open circles) in degrees Celsius, salinity (open triangles) in parts per thousand, and abundance (solid circles) in numbers of fish per seine haul, serially averaged by two's, from June 1967 to October 1968.

Growth

Growth data from Hog Island for young pinfish are presented in Figure 3 (open triangles). Also shown are growth curves from five other studies; two from Cedar Key, Florida (Reid 1954, crosses; Caldwell 1957, squares), one from the Laguna Madre, Texas (Hellier 1962, circles), one from Beaufort, N. C. (Hildebrand and Cable 1938, solid triangles) and one from another area of Redfish Bay, Texas (Hoese and Jones 1963). The data from Hellier and Caldwell represent single year-class; the data from the present study represent two year-classes (lumped together since no difference was apparent). For Reid's study, the two year-classes were not combined since they appear to describe different curves. From Beaufort, N. C., a single year-class is shown. Four of the curves are taken from standard length-frequency histograms and Hildebrand and Cable's data were converted to standard lengths by the formula: std. length = (0.78) × total length.

The relationship between length and weight for 135 pinfish from Redfish Bay is given by:

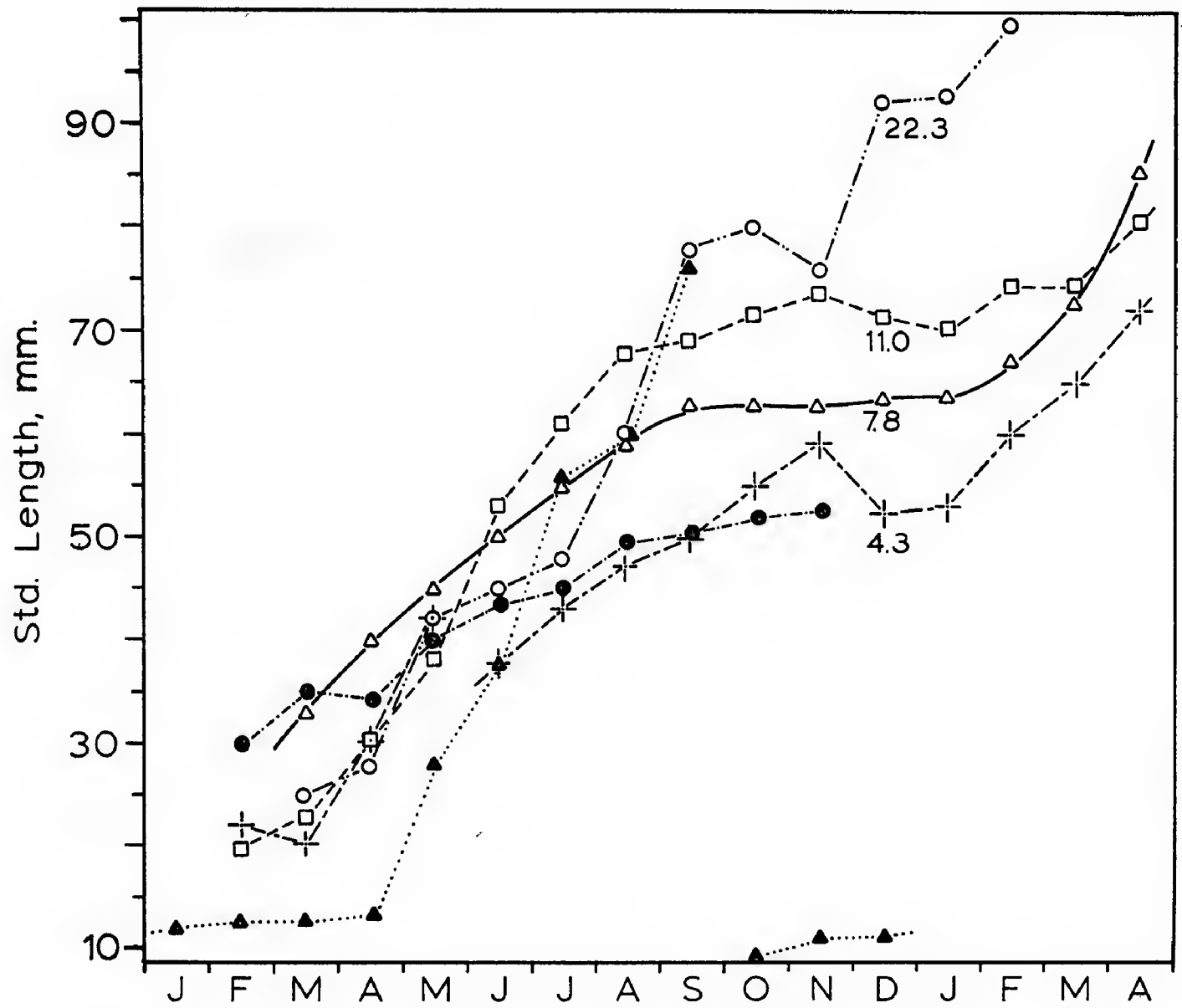

FIG. 3. Growth curves from length-frequency data for the following studies:
Open circles: Hellier 1962, Laguna Madre, Tex.
Closed triangles: Hildebrand and Cable 1938, Beaufort, N.C.
Open squares: Caldwell 1957, Cedar Key, Fla.
Closed circles: Hoese and Jones 1963, Redfish Bay, Tex.
Open triangles: Present study, Redfish Bay, Tex.
Crosses: Reid 1954, Cedar Key, Fla.
The numbers below each point for December are the corresponding weights calculated as described in the text.

$$\log W = -4.353 + 2.903 \log L, \tag{5}$$

where $L =$ standard length in mm and $W =$ weight in gm. The corresponding equation given by Caldwell (1957) is:

$$\log W = -4.373 + 2.914 \log L. \tag{6}$$

The correlation coefficient for equation (5) is $R = 0.99$. Using the appropriate equation, the weight of pinfish at the end of the first year for the four growth curves shown in Figure 3 (using December values) are: Reid—4.3 gm; present study—7.8 gm; Caldwell—11.0 gm; and Hellier—22.3 gm. Even if the November values for Hellier (12.8 gm) and Reid (6.1 gm) are used, there is still a two-fold difference in weight attained at the end of the first year by the same species from these different areas and years.

TABLE 1

Regression equations for pinfish metabolism showing the number of determinations (N), temperature range of the measurements (X_t) and weight range (X_w)

Spring:	$Y = -0.506 + 0.803\,X_w + 0.016\,X_t$	$N = 24$ $X_t = 17$–23 C $X_w = 0.183$–1.543
Summer:	$Y = -2.026 + 0.732\,X_w + 0.071\,X_t$	$N = 18$ $X_t = 25$–32 C $X_w = 0.420$–1.346
Fall:	$Y = -1.865 + 1.132\,X_w + 0.061\,X_t$	$N = 19$ $X_t = 18$–24 C $X_w = 0.462$–1.179
Winter:	$Y = -2.366 + 1.191\,X_w + 0.135\,X_t$	$N = 16$ $X_t = 7$–12 C $X_w = 0.362$–1.295

Where: Y = log (mg O_2 consumed/hr)
X_w = log wet weight in gm
X_t = temperature in Celsius

Metabolism

Multiple regression equations for metabolism, weight and temperature are given in Table 1, along with the numbers of fish used for each equation; also shown are the temperature ranges over which each seasonal set of measurements was carried out. Statistics for the regressions are given in Table 2, including the overall correlation coefficients, partial regression coefficients in standard measure, their standard errors and the probability estimates for each.

Regression lines for fish of two different weights, 5 and 30 gm, are plotted against temperature, as log oxygen consumed per hour, in Figure 4. Each season's data are plotted only over the actual range of temperatures used in the measurements.

One additional set of measurements during summer at 31.7 C has not been included in the regression equations for any season. Means for this group are plotted in Figure 4 (crosses) adjusted for weight. Obviously, this group would not be suitable for inclusion in the linear temperature approximation for the summer

TABLE 2

Regression statistics for equations in Table 1. Values for s_b' represent standard errors of the partial regression coefficients in standard measure, and standard partial regression coefficients are given (b_w' and b_t')

Season	N	$Ry{\cdot}tw$	P	s_b'	b_w'	P	b_t'	P
Spring	24	0.97	<0.001	0.059	1.019	<0.001	0.121	$0.05<P<0.10$
Summer	18	0.96	<0.001	0.069	0.673	<0.001	0.486	<0.001
Fall	19	0.87	<0.001	0.160	1.149	<0.001	0.765	<0.001
Winter	16	0.87	<0.001	0.188	0.603	<0.01	1.072	<0.001

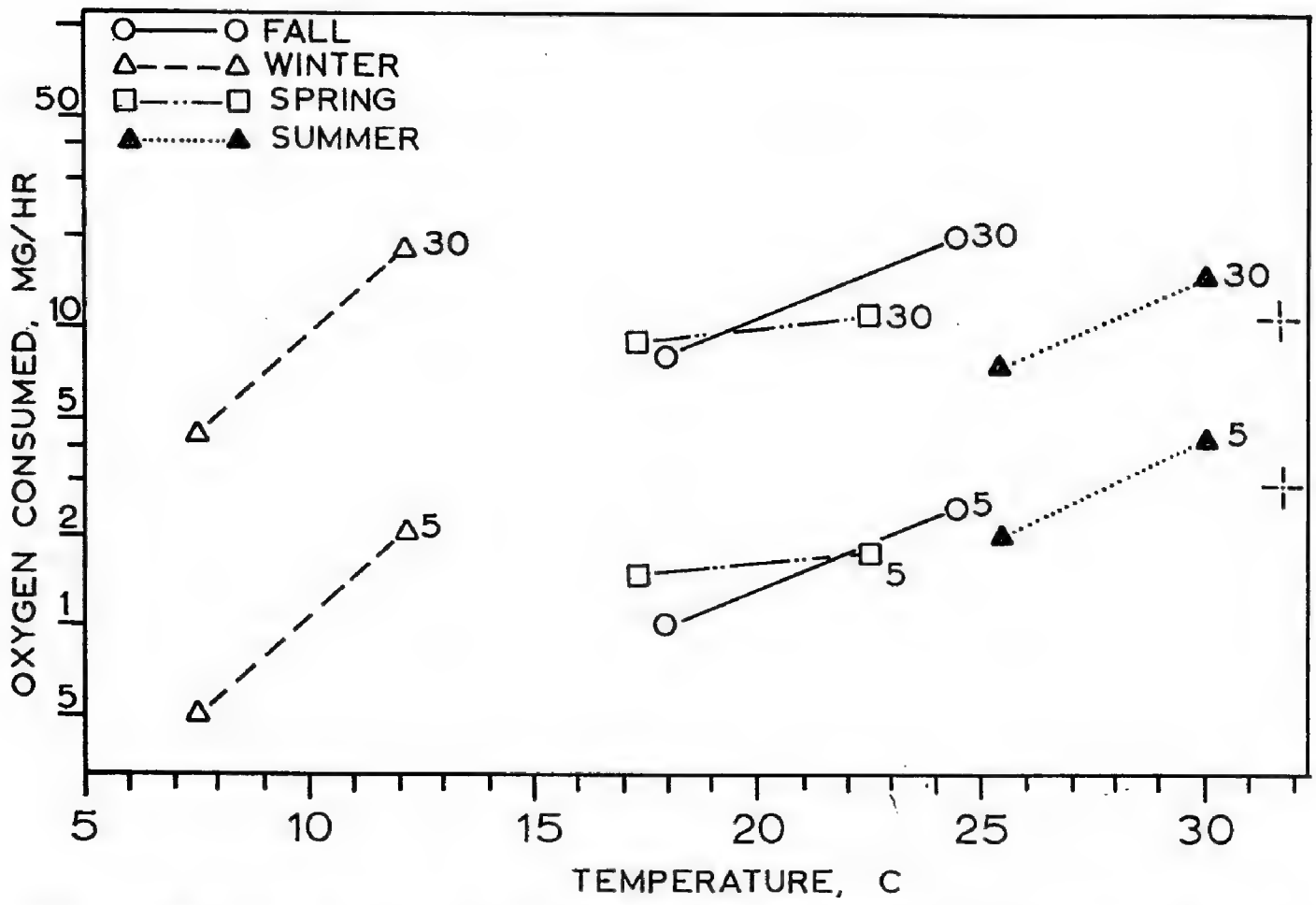

FIG. 4. Metabolic rates of 5 and 30 g pinfish at different seasons, calculated from the regressions on weight and temperature for each season (see text). Each season is plotted only over the actual range of the field measurements. Oxygen consumption is in mg/hr. Crosses indicate weight-adjusted rates for one series at 31.7 C.

season, since they show a marked drop from what would be expected on the basis of results extrapolated from the other summer data.

As an indication of the changes in respiratory demand from one season to another, taking into account differences in weight, temperature and seasonal temperature adaptation, the following calculation was made: average weight and temperature for each month were converted, with the regression equation appropriate for that season, to predicted oxygen consumption values for a hypothetical year class. This was done for data from February 1967 to April 1968 and the result is shown in Figure 5. By multiplying these oxygen consumption values by the density of pinfish in any given habitat, an estimate of the respiratory demand of that population would be obtained.

DISCUSSION

Seasonal Distribution

Although the pinfish has never been observed during spawning and only a handful of ripe individuals has now been recorded (Caldwell 1957; Springer 1957), it is clear that spawning takes place in the late fall and early winter. Postlarval pinfish have been taken from October (Hildebrand and Cable 1938) to the end of April, with a peak generally coming in December to February (Hildebrand and Cable *op. cit.;* Caldwell 1957; Hoese 1965).

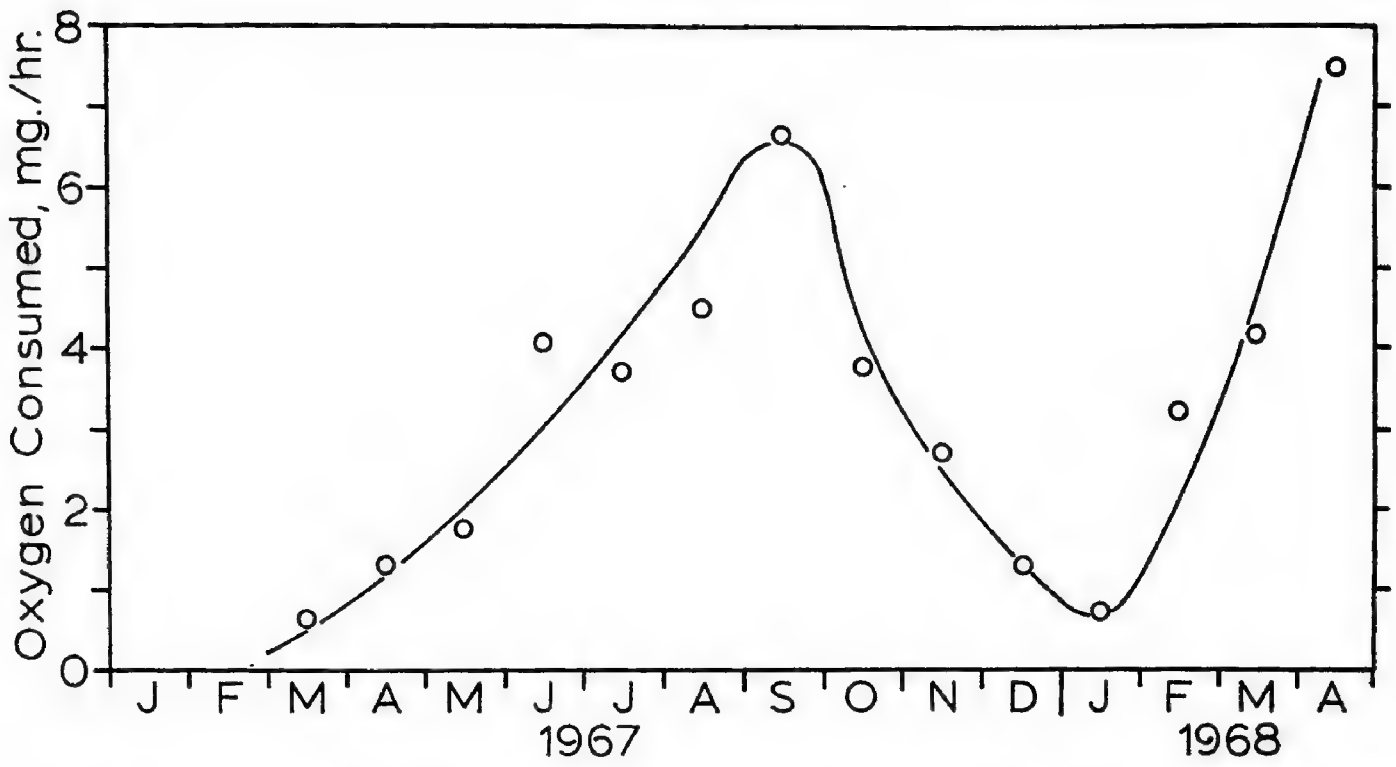

FIG. 5. Metabolic rate of the average size pinfish for each month from March 1967 to April 1968. Figures calculated from average size, temperature and seasonal regression equation for each month, as described in text.

Spawning probably takes place well offshore. Hildebrand and Cable (*op. cit.*) took larval forms at their most distant offshore stations (12 to 13 miles) and Hoese (*op. cit.*) took them as much as 10 miles offshore in Texas. In both cases, the larval fishes were up to 10 mm in length already, indicating that they could have come from much farther offshore.

The migration of the postlarval pinfish from offshore to inshore and bay areas can be traced from the dates of appearance in collections made in the various areas. Hildebrand and Cable (1938) report that postlarvae were numerous in deeper channels around Beaufort during the cold months of the winter. Hoese (1965) reports that they appeared inshore on December 10, 1962 in Texas. Apparently the influx continues through April, the larger specimens reaching the shallow vegetated areas of the bays by February, where they remain for some time.

Samples from the grass flats reflect this pattern of movement, showing increasing abundance in the early spring (Fig. 2; Hoese and Jones 1963; Hellier 1962; Caldwell 1957). A peak is reached in the spring, after which populations decline at varying rates in the samples from all areas studied. The data from Hoese and Jones (*op. cit.*), however, shows an interesting difference when compared either to the other studies cited or to the present study. Pinfish reached two peaks of abundance in the Hoese and Jones study area, the usual one in the spring, but also another peak in fall, following a severe depression in abundance in midsummer. The absence of pinfish on at least one summer date on the present study area has also been mentioned.

Hoese and Jones' area is the shallowest of all the areas being compared and, further, it is isolated from any deeper channel either by spoil banks or by extensive flats (Hoese and Jones 1963, Fig. 1). This isolation means their area is subject

to more rapid and more extreme fluctuations in environmental factors, particularly temperature, than the others. It also means that the movements required to escape these conditions must be correspondingly longer and metabolically more costly. Data for the present study were taken from an area close to a deeper, more stable channel, whereas data for the other studies of pinfish abundance and distribution were either taken from deeper areas (Hildebrand and Cable 1938; Hellier 1962) or presented in such a manner that the abundance in deep and shallow areas could not be compared (Reid 1954; Caldwell 1957). Temperature data are presented for three of the studies besides this one and, again, the most extreme temperatures occurred in Hoese and Jones' study (10 to 35 C) and the present study (7.6 to 35 C).

At temperatures over 31 or 32 C, the abundance of the pinfish on Hoese and Jones' study area was low. At these temperatures in the present study it was zero. Tolerance limits for the pinfish from Redfish Bay have been approximately determined to be 32 to 33 C (Cameron 1969). Apparently, then, the pinfish is responding to a potentially lethal temperature stress by migrating to deeper, cooler areas; temporarily in the case of Hog Island and perhaps for longer periods on Hoese and Jones' area. The energy necessary for daily movement to and from Corpus Christi Bayou would be far less than daily movement from Hoese and Jones' study area to a comparable deep area, which probably accounts for the observed difference in the pattern of abundance in the two areas. It is indeed unfortunate that no night samples were taken by Hoese and Jones to see if daily migrations were occurring.

In addition to the movements of juveniles from offshore waters in spring onto shallow flats, there is a marked tendency for larger fish to move gradually into deeper waters. Caldwell (1957) discusses this in some detail for Cedar Key and data gathered in the present study also show a size-depth correlation. Various methods of fishing were employed on one occasion, including hook and line, trawls and seines, at various distances from Hog Island in the easterly direction of Corpus Christi Bayou. The closer to the bayou, and the deeper the water, the bigger the pinfish that could be caught. No pinfish over 100 mm were caught in less than 1 m of water, and few pinfish under 100 mm were caught in over 2 m of water (August 1967).

One consequence of movement of larger fish into deeper waters is that growth data from length-frequency analyses are apt to be strongly biased in favor of smaller modes. That is, if smaller fish are being added to the length groups during the first half of the summer, and larger ones continually migrating away from the flats, the resultant growth curve is likely to be an underestimate of the true growth of any individual. This point will be further considered below.

Although this size-depth correlation has been consistently observed, no explanation has been attempted by any author. Darnell (1958) reported that the food habits of the pinfish do change as the fish grows larger. This suggests that changing food requirements may prompt migration of larger specimens to other areas. "Microbottom animals" (Darnell *op. cit.*) may be more abundant in shallow flats, while plant material is more available in deeper areas.

Much of Ivlev's (1961) data shows also that as the size of the fish changes, its

efficiency of gathering small food items changes; so smaller pinfish may be at an advantage in obtaining the predominantly small food abundant on the grass flats. A third possible explanation for the observed pattern of movements of larger fish into deeper waters may be that the larger fish are less able to cope metabolically with extremes of temperature and dissolved oxygen that commonly occur on the flats. This would explain their movement into more stable, deeper areas of the bays, or at least to their margins, as Caldwell (1957) notes.

Less information can be found on the last possibility, namely that metabolic considerations favor movement of larger fish into deeper waters. However, according to the von Bertalanffy (1938) theory of metabolism, the larger the fish, the less metabolic energy it can channel into activities other than normal maintenance (Beverton and Holt 1957). Since the movements of larger fish into deeper waters occur at times of actual or potential temperature stress, such movements may be related to the relative ability of large and small fish to meet the extra metabolic demands necessary to cope with such stress. In this regard, studies should be made of the relationship between size and temperature tolerance, size and metabolic depression at high temperature, and size and tolerance to low oxygen.

Growth

Comparison of the various growth curves in Figure 3 shows some similarities and many interesting differences. For example, differences among the Cedar Key growth curves (Reid 1954; Caldwell 1957) are greater than differences between Cedar Key and Redfish Bay (Hoese and Jones 1963; present study). Further, the two year-classes studied by Reid show widely different growth rates; the second year-class he studied apparently began the spring at a considerably greater size, but the rate of growth appears to be similar. The four curves from Redfish Bay and Cedar Key all show a decrease in the rate of growth toward late summer and fall, and three of these actually show a decrease in mean length for at least one month. On the other hand, the fish from Laguna Madre and Beaufort, N. C. do not show this decrease in rate and apparently continue to grow, although the data from Beaufort do not continue late enough in the season to be definitive.

Actually, presentation of the data as growth in length tends to minimize the differences in the curves. Conversion of the lengths to weights according to the equations given earlier yields a five-fold difference in December weights. Even if November weights are used for Reid's and Hellier's data, which are 6.1 and 12.6 g, respectively, there is still more than a two-fold difference in weight attained at the end of the first year. These differences can perhaps be explained by the following considerations.

Movement of larger fish from the shallow flats to deeper channel and bay areas during late spring and summer has already been discussed. It is significant, then, that the two growth curves which continue to show an increase in length beyond September, namely Hellier's and Hildebrand and Cable's, are both derived from collections made primarily in deeper areas. Hellier gives the average depth of his study area as about 0.8 to 1.2 m, and also includes several stations adjacent to channel areas. Hildebrand and Cable do not state the depth of their area but on

their map most stations are 3 m or more in depth. It therefore seems likely that the gradual movement of larger pinfish into these deeper areas has resulted in the continued increase of the growth curves shown.

The remaining growth curves are from shallow flat areas, from which the larger fish are migrating. It is not surprising that these areas will show a decrease in growth rate as the larger fish leave. The effect of migrations and the location of sampling stations is to produce growth curves biased upward for deeper areas and downward for shallow flats. The true growth rate of individuals in the population probably lies somewhere in between.

A further peculiarity shown by the growth data from Hildebrand and Cable (1938) is the presence of very slow-growing juveniles during the cold, winter months, from October to April. Their discussion of these data implies that these fish were collected from channel areas receiving migrating postlarvae. Since the spawning season is extended over several months, what they were probably sampling were not the same individuals, which grew very slowly, but a continually renewed population of incoming juveniles, which were of approximately the same size and age when sampled. Therefore, the true rate of growth of the fish in their area was probably greater than indicated by the winter data and probably slower than shown in the spring data.

Besides differences due to sampling area and time, a further difference is shown by Reid's data from one year to another. The later year-class (left hand crosses, Fig. 3) apparently had reached a larger size at the time of appearance on his study area. This was probably due to a somewhat earlier spawning season, or higher winter temperatures leading to greater winter growth. Insufficient data are available for any further consideration of this point. Such year-to-year differences were not apparent in the present study and the curve presented is the result of combining data for two year-classes.

The decrease in mean size that appears in the second winter in three of the curves (present study; Caldwell 1957; Reid 1954) may or may not represent an actual weight loss by any individuals. It may simply be the result of a decrease in the average of the population due to egress of the larger members.

Despite differences in rate and duration of growth, data from all areas show similar timing of major events, which is undoubtedly due to the similarity of the grass associations in these areas. During the warming period in spring, the grasses develop into a thick carpet over very extensive areas. Many juvenile fish and invertebrates take advantage of the food and cover afforded by the dense stands throughout the spring and summer. However, after blooming, the grasses die off in large quantities in the fall, persisting only at low densities through the cold temperatures and reduced light conditions of the winter. The spawning and rapid growth periods of the pinfish are apparently geared to the development of the grasses.

Metabolism

Metabolic data now have been published for a wide range of species and much of the material has now been exhaustively reviewed (Winberg 1956; Fry 1957, 1964). The data shown for the pinfish are roughly comparable to other temperate

or subtropical fishes of moderate activity and show similar responses to weight and temperature, both within seasons and over the year as a whole. Cold adaptation occurs in the pinfish (*cf.* Scholander *et al.* 1953), since the metabolism-temperature curve is shifted substantially upward in winter from what would be expected on the basis of an extrapolated summer curve.

There are, however, some interesting differences between the normal metabolic situation and the pinfish data. Metabolism-weight coefficients are generally on the order of 0.67 to 0.8 (Wohlschlag 1964, Fig. 7) for fishes and other organisms (Prosser and Brown 1961), but the fall and winter metabolism-weight coefficients for pinfish exceed the usual range. Similarly high metabolism-weight coefficients have been described also by Wohlschlag (1963, 1964) for several Antarctic fishes. He suggested that they were caused by "excess upward variability" of the larger fishes due to increased irritability and activity. Anything tending to increase the metabolism of the larger fish relatively more than the smaller ones would lead to such metabolism-weight coefficients, so this might be one possible source. Another possibility is that the size of the container relative to the size of the fish caused an increase in irritability in the fall and winter runs that was not present in the other seasons.

The metabolism-temperature response coefficients for the spring, summer and fall were within the limits reported for fishes and correspond to Q_{10} values of 1.5, 5.1 and 4.1, respectively. The spring value is rather surprisingly low. Since the measurements were made over a period of several weeks when temperatures were rising, progressive adaptation to higher temperatures could have occurred, leading to low apparent temperature response. On the other hand, calculation of the between-season, or overall Q_{10} value yields 1.7, showing a remarkable degree of temperature compensation over the whole year. The biological value of such effective compensation for a fish inhabiting such a thermally unstable environment is obvious.

The b_t value of 0.135 for winter ($Q_{10} = 22$), however, is well above the normal range of values for biological reactions and in fact more nearly resembles the range reported for thermal death (Giese 1962). Since the lower lethal limits have been determined in our laboratory to be between 6 and 8 C (Wohlschlag and Cameron, unpublished data) for winter-acclimated fish, it should not be surprising that metabolic determinations over the range from 7 to 12 C would show a large temperature response. It is also interesting to note that when temperatures reached 7 C, the pinfish could only be collected from Corpus Christi Bayou, which was 1 to 2 C warmer than the grass flats. It appears, then, that the metabolic depression shown by the regression for winter was closely related to distributional shifts observed in the field population.

In that regard, the set of metabolic data that was not included in the summer regression (see Fig. 4) showed a depression setting in at temperatures over 30 C. In fact, at temperatures over 32 C, no pinfish could be collected from their normal habitat near Hog Island, though they were there on cooler days and during the night. At the upper limits of their temperature range, then, there is also a relationship between metabolic depression and distributional shifts.

Comparison of standard partial regression coefficients in Table 2 also reflects

TABLE 3

Comparison of statistics for pinfish metabolism

Statistic	Present study	Wohlschlag *et al.* (1968)
Spring b_t	0.016	0.039 (10–20 C)
		0.016 (20–30 C)
Summer b_t	0.071	0.018 (20–30 C)
Fall b_t	0.061	0.028 (10–20 C)
		0.015 (20–30 C)
Winter b_t	0.135	0.029 (10–20 C)
Spring b_w	0.803	1.004 (10–20 C)
		0.789 (20–30 C)
Summer b_w	0.732	1.032 (10–20 C)
		0.818 (20–30 C)
Fall b_w	1.132	0.972 (10–20 C)
		0.838 (20–30 C)
Winter b_w	1.191	0.939 (10–20 C)
		0.786 (20–30 C)

the relative importance of temperature at different times of year. In winter, temperature appears to have a greater influence on the metabolic rate than weight, but in summer an approximately equal influence. In fall and spring, however, temperature is less important, but the metabolic rate is most effectively temperature compensated in spring.

A comparison of the data given in the present study and that given for larger pinfish in somewhat different circumstances by Wohlschlag, Cameron and Cech (1968) reveals that the peculiarities of the data from Hog Island do not appear in their data. For a variety of reasons, the absolute levels of metabolism cannot be compared for the two studies (mainly due to differences in methods), but there is no reason why the responses (b-values) to weight and temperature should not be comparable. Such a comparison is made in Table 3.

Although it is generally the case that the b_t's for the higher temperatures in Wohlschlag *et al.* (*op. cit.*) are lower than for low temperatures, no increase in temperature response was noted for summer and the b_t values for winter were not nearly so large. This is probably at least partly due to more extreme temperatures included in the present study. Additionally, in the former study the fish were held at a constant temperature prior to testing, whereas the fish in the present study in most cases had probably been exposed to the test temperature for a maximum of a few hours.

High weight coefficients also occur in the former study, but they are not confined to any particular season. Whether some behavior pattern was present in the fish used for this study is not known, but the high weight coefficients occur in both studies only at the lowest test temperatures.

SUMMARY AND CONCLUSIONS

The patterns of growth and abundance reported for pinfish from widely different parts of their range show some marked differences in growth rate and sea-

sonal abundance. These differences can be reconciled partly on the basis of migratory behavior in relation to depth and location of the study areas. Thus, the summer depression of abundance in Hoese and Jones' (1963) study is likely due to the absence of any readily available refuge from extreme conditions occurring in that area. Temperatures reported for that area are well above the lethal limits determined for pinfish (Cameron 1969) and above the point at which metabolic depression is evident. Similarly, differing patterns of growth from the Laguna Madre, Texas (Hellier 1962) and Beaufort, N. C. (Hildebrand and Cable 1938) can be explained on the basis of depth; these areas are deeper than others studied (present study or Reid 1954). Caldwell has discussed the movements of larger fish from shallow into deeper areas, and the same pattern of movement was observed in the present study. It appears likely, then, that the increased autumn growth shown for Laguna Madre and Beaufort is due to the continued arrival of larger fish into the deeper areas sampled.

On the other hand, continued influx of postlarvae and small juveniles from open waters and channels will tend to bias the growth curves obtained from collections in shallow flat areas. The growth rate of individual fish undoubtedly lies somewhere between the extremes represented by the two groups of data.

In all cases studied, the influx of young fish is timed to the beginning of growth of the grasses that dominate these flats and coinciding with the arrival of many other species of fishes and invertebrates (Hoese and Jones 1963). Winter spawning in the pinfish is probably an adaptation to the utilization of this habitat by the young.

After the period in spring and summer when the juveniles show their greatest abundance and growth the abundance declines markedly, leaving only a small residual population on the flats over winter. On certain occasions, even this small component is absent when temperatures drop rapidly to very low levels. The deeper channels and bay areas adjacent to the flats provide a warmer, more stable refuge from environmental extremes, and here pinfish can be found in large numbers. The migrations involved in withstanding winter conditions undoubtedly contribute to the low growth and abundance of the pinfish at this season. Severe metabolic depression is also evident at temperatures comparable to those measured at the time the fish were absent.

There are still many unanswered questions, such as why the larger pinfish tend to seek deeper waters or how frequent and extensive are the temporary migrations from shallow to deep areas. However, the metabolic limitations operating at high and low temperature extremes undoubtedly provides at least a partial explanation of why the pinfish does not appear on the flats in any numbers until the water begins to warm and why, in some areas at least, the abundance data show a secondary low period in mid-summer. A further investigation of some of these problems, in which there are both the movements and physiological responses of the population and individuals, should provide some of the answers.

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A PORTABLE DROP-NET FOR REPRESENTATIVE SAMPLING OF NEKTON[1]

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ABSTRACT

A portable sampling device has been developed to representatively sample the nekton standing crop on an areal basis in shallow estuarine systems. The pursing net is suspended onto a floating frame with electro-magnets and samples an area of 16 square meters. The device is portable so that replicate sampling can be done to obtain statistically reliable data.

INTRODUCTION

One of the more difficult problems in studies of nekton populations has been that of obtaining representative samples. Several methods have been used, but most of them have limitations. Methods developed in the past, such as tagging and clipping (DeLury 1951, 1958), determinations of catch per unit effort (Carlander 1956; Ricker 1948), electrical sampling (Haskett 1940) and draining or poisoning small areas (Stetter and Leonard 1943), are limited to being effective only for specific organisms or for local situations. Recently, however, the development of the quadrate sampling by suspended nets (Hellier 1958, 1962; Hoese and Jones 1963), helicopter-borne purse nets (Jones *et al.* 1963; Jones 1965) and a

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demersal animal sampler (Van Cleve *et al.* 1966) has improved the possibility of representative sampling. All of these methods, unfortunately, have serious drawbacks. The suspended nets are necessarily limited to one place, thus after continuous use in the same area, deterioration of the bottom occurs and limits their effectiveness. They also require seining the enclosed area, which places some bias on the reliability of the sample since not all of the enclosed nekton can be captured. The helicopter-borne purse net and the demersal animal samplers require large and expensive equipment to implement the drop, which limits them to waters deep enough to accommodate large vessels.

There is a need for a portable quantitative sampling device that will not only accurately sample the existing nekton community, but is useable in shallow estuarine waters and is completely portable for multiple sampling that is necessary for statistical verification of population size. This paper deals with the description of a portable drop-net that has been used in the Guadalupe River-San Antonio Bay estuarine complex and the Mission-Aransas Rivers-Copano Bay estuarine complex.

NET DESCRIPTION AND OPERATION

Description of Net and Support Frame

The sampling technique involves surrounding a known area of water (in this case, five meters × five meters) with a small-mesh purse net (Fig. 1). The net is composed of nylon webbing (1 cm stretch) and is five meters × five meters × five meters deep, with an enclosed top of the same webbing. It is weighted at the bottom with heavy chain (1.5 kg/m) and the top is prevented from sinking by light floats attached to the edges. The heavy chain-line penetrates the soft bottom a few centimeters, as evidenced by the capture of burrowing organisms. Due to the weight of the chain, the bottom of the net collapses inward resulting in an effective sampling area of 16 square meters.

The frame (five meters × five meters) is constructed from heavy lumber and is floated by eight 55-gallon barrels (Fig. 1). The weight of the net and frame causes the floats to sink only about 15 cm in the water, thus being useable in most shallow estuarine areas. The floating frame (with net) is easily towed by outboard motorboat to various sampling places.

Operational Techniques

The net is attached to the frame with 16 electro-magnets wired in series (Figs. 1 and 2), which are supported by a 36-volt rechargeable battery supply. When in the pre-drop position the net is supported about one meter above the water level by attaching the chain-line at regular intervals to 12 of the electro-magnets (*see* Fig. 1). The top of the net is supported at each corner by the remaining four electro-magnets. The net is suspended in the above manner and the floating frame is then positioned in the desired sampling area. When the net is ready to be dropped, a reverse current (provided by an auxiliary battery) of six volts is passed through the circuit to cause the electro-magnets to release the net simultaneously and instantaneously.

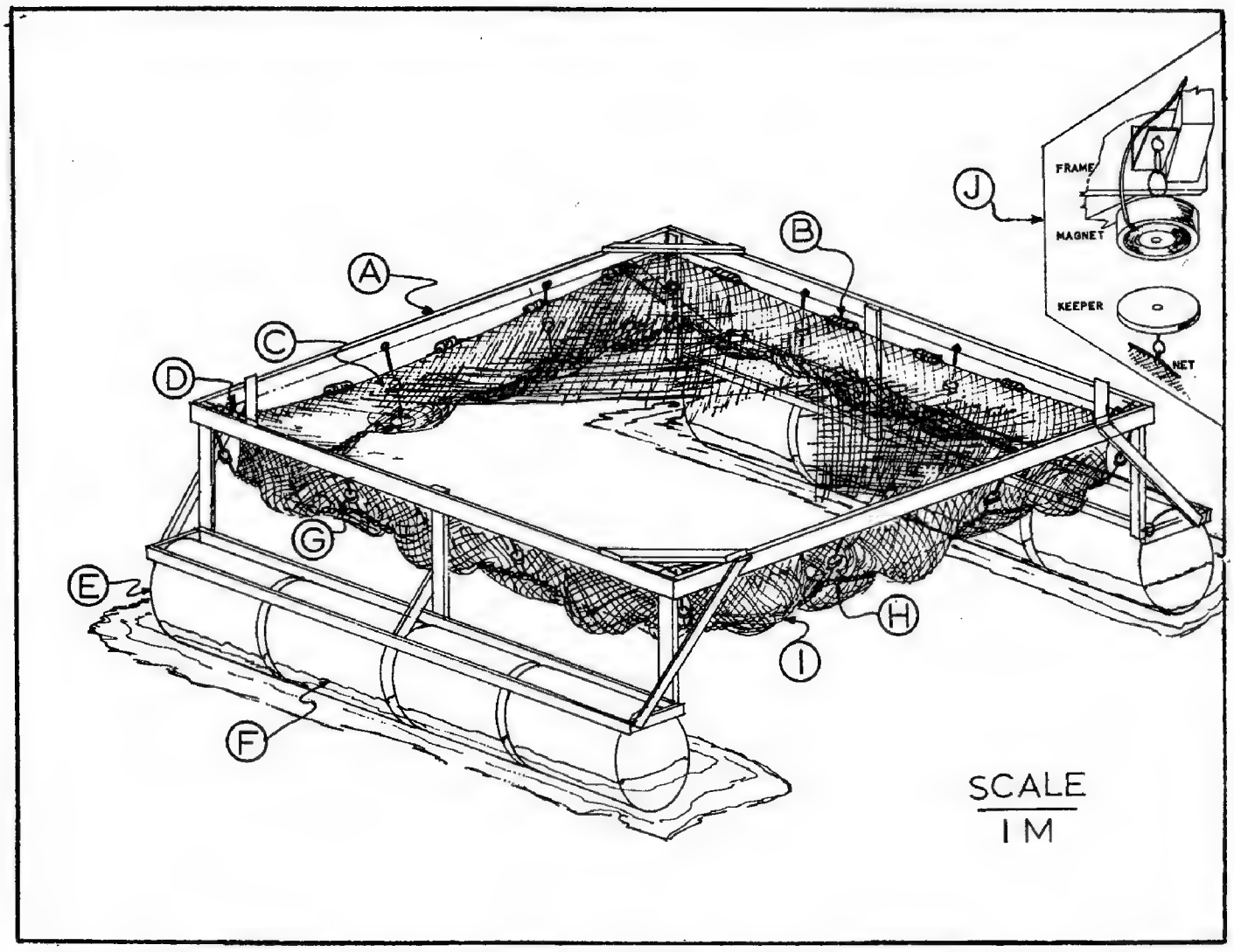

FIG. 1. Diagram of the floating frame and portable net in the pre-drop position. A. Support frame. B. Floats attached to top edges of the net. C. Top of the net. D. One of four electro-magnets to suspend top of the net in pre-drop position (one at each corner). E. One of eight floats to support the frame. F. Water line. G. One of 12 electro-magnets to suspend the chain-line of the net in the pre-drop position. H. Chain-line. I. Wall of the net; tucked over the chain-line during the pre-drop position. J. Inset showing the details of the electro-magnets.

A detailed diagram of an electro-magnet and magnet circuit is given in Figure 2. The magnet cup was made by machining a circular groove in a piece of stock steel and winding 200 coils of wire inside it (*see* Fig. 2A and B for dimensions and general configuration). The keeper, which is attached to the net, was cut from the same stock. Eye bolts, screwed into both the magnet and keeper, facilitated attachment to the frame and net, respectively. The switch, shown in the circuit diagram in Figure 2C, was three-way so that all circuits could be disconnected when the sampling device was not in use. By lengthening the wires between the switch and remaining circuits, drops could be done by remote control.

The weight of the heavy chain causes the net to drop extremely fast, thus lessening the chance for the entrapped organisms to escape. The escape of organisms is theoretically nil, since the flight from the sound of the falling chain-line should be in a random direction. Never have we observed organisms escaping when the drop is properly done.

After the net is dropped from the frame, with the chain-line snugly on the bottom and the top floated by plastic floats, the pursing operation is begun (Fig. 3). A pursing line, which is passed through a series of rings attached near the bottom

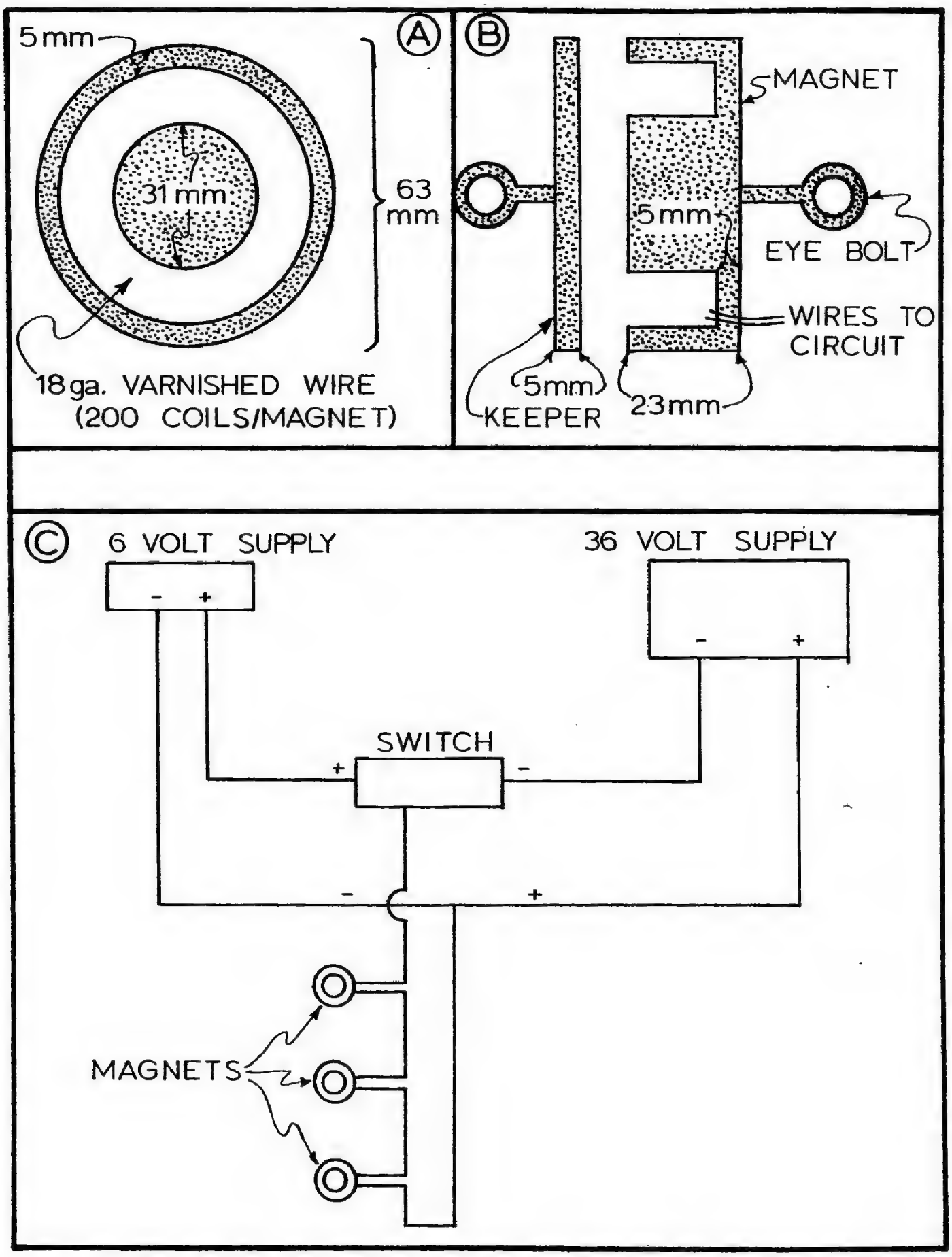

FIG. 2. Diagram of the magnet assembly and wiring circuit. A. Bottom view of the magnet, giving dimensions and winding. B. Sagittal view of the magnet and keeper. C. Magnet wiring circuit, giving position of the two battery supplies (see text for explanation; 16 magnets in series).

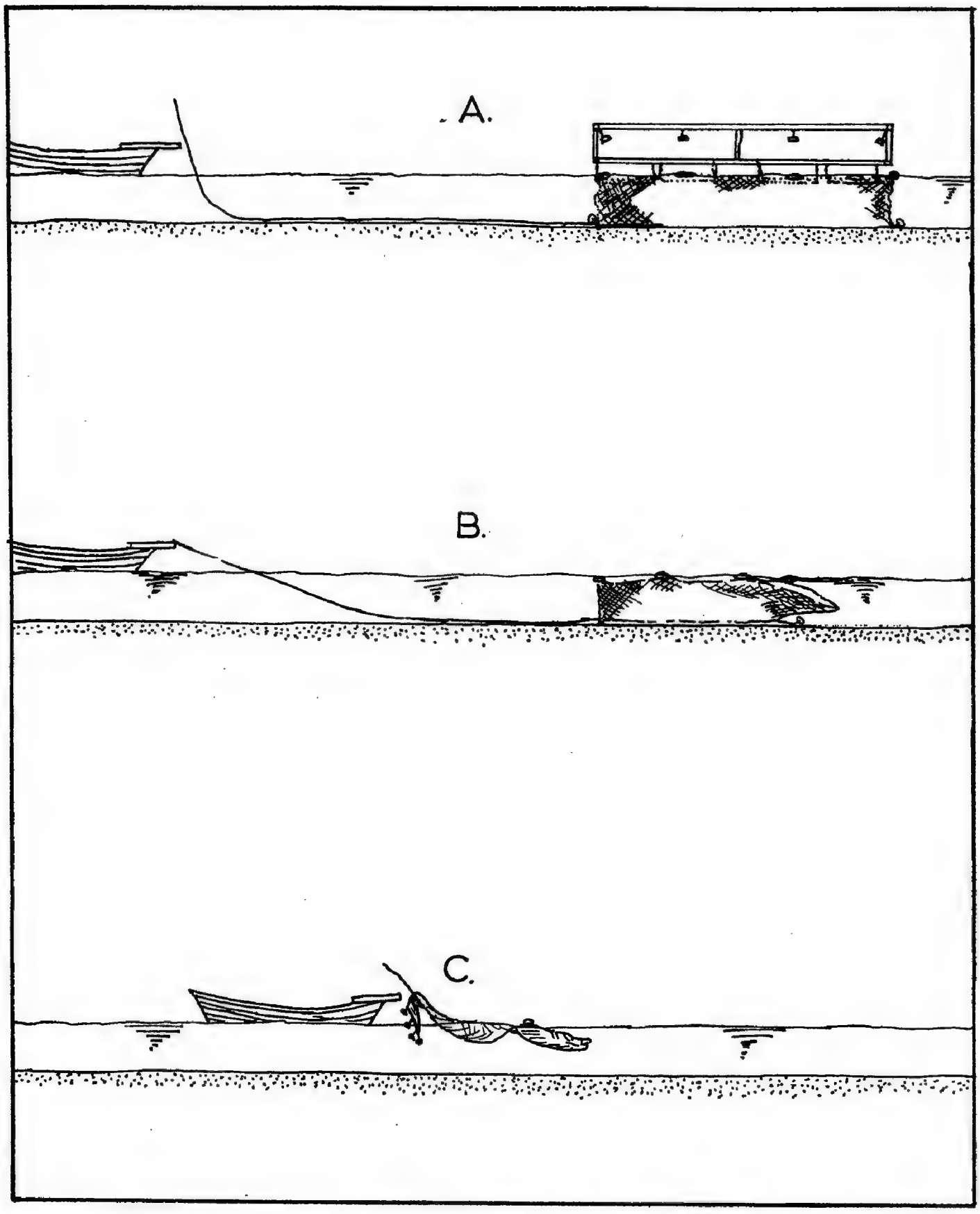

FIG. 3. Sequence of pursing and retrieving the net after a drop. A. Net in the water, chain-line on bottom and the pursing line attached to the boat for the pursing operation. Note that the net is directly underneath the frame. B. Pursing the bottom of the net. Note that the pursing line is long enough to enable pursing without lifting the chain-line from the bottom. C. Bottom of the net pursed and net ready to be hauled aboard the boat.

around the periphery of the net, is attached to a small boat for the pursing procedure (Fig. 3). The pursing line is long enough (65 meters) to surround the net and allow enough distance between the boat and net to purse the net without lifting it off the bottom in the shallow water. By pulling the purse-line with the

boat the net is pursed and the captured organisms pushed to the enclosed top (Fig. 3B). After the net is completely pursed, it is lifted from the bottom and hauled aboard the boat (Fig. 3C). The net is carried to shore where it is spread and the organisms collected.

EXAMPLE OF RESULTS

To show the applicability of the sampling device, total weight of nekton in gm/m^2 captured in Guadalupe Bay, Texas during one year's sampling is presented in Figure 4. The vertical lines indicate the variation between day and night samples.

The sharp peaks in biomass concentration are attributed to the local abundance of young menhaden, *Brevoortia patronus*, which are migrating forms in this system. Not only were there variable concentrations of young menhaden between sampling dates, but considerable variation between day and night samples within the same date. This is not surprising in view of the fact that young menhaden, when in the upper bay areas, tend to congregate in moving schools of several thousand individuals or more. Menhaden were present in the sampling area during March through August, which corresponds in time with the findings of Jones (1965) from a helicopter-borne drop-net sampling of Corpus Christi Bay.

During the winter, the nekton population was seemingly constant in Guadalupe Bay, because variation among catches between dates and within dates was relatively small. On the other hand, during spring and summer, the nekton popula-

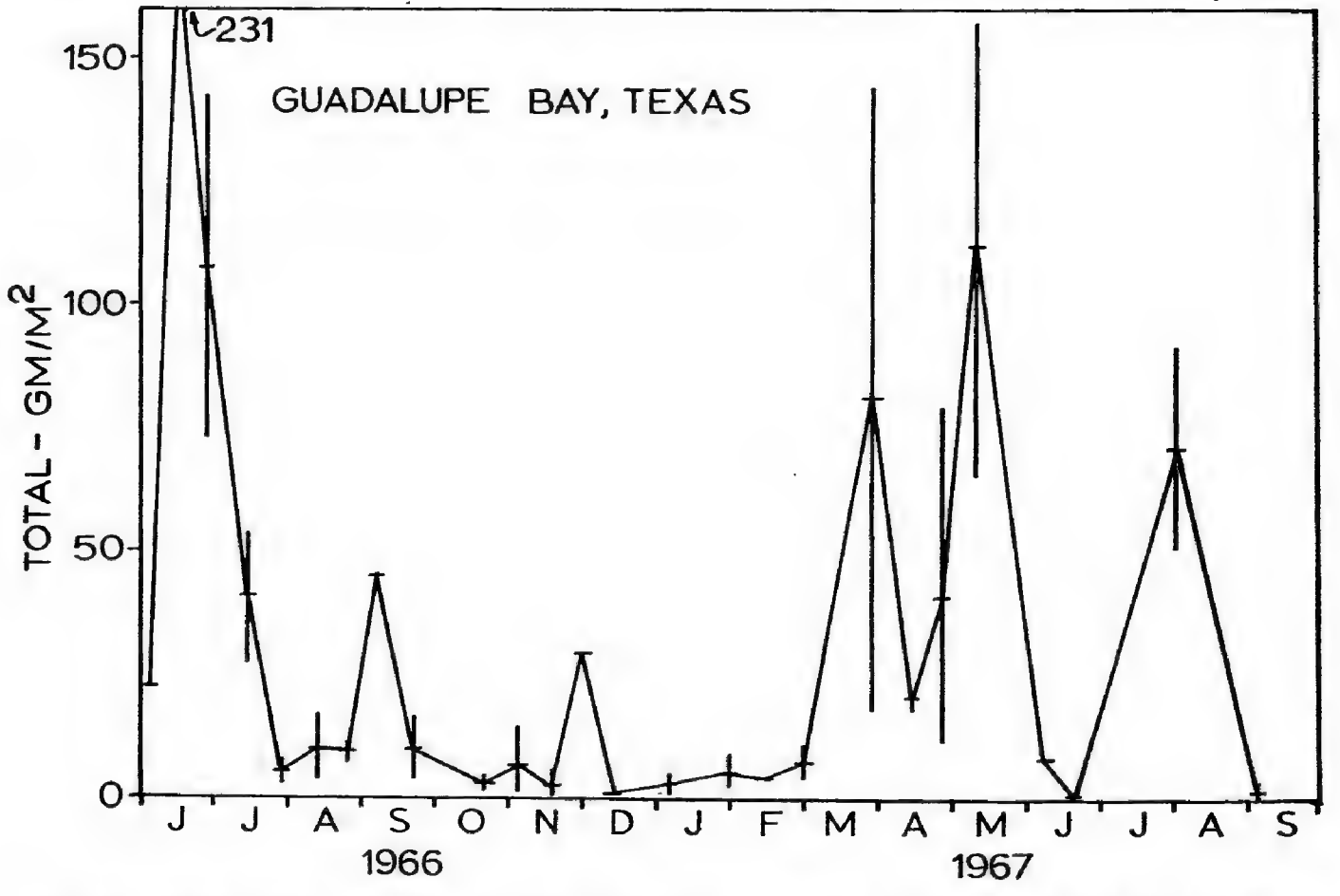

FIG. 4. Total catch with the portable drop-net (illustrated in Figs. 1, 2 and 3) during one year's sampling program in Guadalupe Bay, Texas. Each point represents the mean of two drops (one night and one during daylight) and the vertical line indicates the range of values.

tion in Guadalupe Bay was quite variable, indicated by the relatively large variation among catches between dates and within dates. The large variation within a date of sampling (the vertical line in Fig. 4) may be due to schooling and is also attributable to differences in day and night catches, as pointed out by Hoese *et al.* (1968). Indeed, the largest biomass per square meter almost always occurred in the night samples, which may indicate bias by shadow effect and high winds during the day.

SUMMARIZATION OF APPLICABILITY

The net described above is not without its limitations, but it offers many advantages in quantitatively sampling nekton as compared to conventional gear. The relative advantages and disadvantages are presented below.

Advantages

Use in Obstructed Areas—The drop-net can be used in shallow stream and estuarine areas where gear such as trawls and seines may be torn during usage and where motorboats large enough to tow traws could not be taken. The drop-net was successfully used in the Guadalupe, San Antonio, Mission and Aransas Rivers where other types of gear usage would have been impossible. We also sampled shallow estuarine areas in Guadalupe Bay (see the results reported in Fig. 4), where the water depth was less than one meter. By dropping the net between submerged logs and other debris, we quantitatively sampled the nekton in the lower San Antonio River.

Organisms not Usually Taken by Trawl—Organisms were collected with the drop-net that are not ordinarily taken in trawls and seines, such as near-surface swimmers like *Brevoortia patronus*, *Mugil cephalus*, *Lepisosteus spatula* and *L. osseus*. The collection of flounders (*Paralichthys*), penaeid shrimp, midshipman (*Porichthys*) and rays (*Dasyatis*) indicates that bottom dwellers and burrowers are also readily taken. Thus, the drop-net samples yield more representative data concerning the areas sampled than do samples taken with more conventional gear.

Protability and Remote Control—The floating frame, with its attached net, is easily towed about behind a small outboard skiff to remote areas and in very shallow water. By pre-suspending the net and then towing the frame to a study area, minimum disturbance of the sampling area is assured. Utilizing a long lead between the magnet circuit and the device to trigger the drop, the net can be dropped by remote control without disturbing the immediate sampling area. The compactness of the apparatus lends itself to smaller craft and manpower requirements than the quantitative devices proposed by Jones *et al.* (1963) and Van Cleve *et al.* (1966). Its portability allows versatility as well as the prevention of sampling site destruction as in the case of devices proposed by Hellier (1958) and Hoese and Jones (1963).

Representative Samples—The most obvious advantage, of course, is the ability to obtain representative samples for specific areal expanses. Representative data

(such as those shown in Fig. 4) are essential for obtaining meaningful data for studies of productivity, structure, standing crop, etc., for estuarine ecology.

Disadvantages

Small Sample Size—Because of the compactness and portability of the net, the effective sampling area for any one drop is only 16 square meters (measured by the circumference of the chain-line after being dropped). This small sample area probably is representative of small and more numerous organisms, but may yield biased results from larger and less abundant organisms. Never were large trout or redfish taken in over 300 drops during a two-year period, although a few large specimens were known to be in the area during part of that time. Large gar (*Lepisosteus spatula* and *osseus*) were taken on occasion and they were believed to be much more numerous in the sampling areas than either trout or redfish. Therefore, this method may not be efficient for sampling some large organisms in certain estuarine areas.

Small Number of Samples—The time required to set, position, purse and empty the drop-net is considerably greater than that required for samples taken by trawl or seine, thus yielding fewer samples per unit time and effort. Utilization of other quantitative devices such as the helicopter-borne purse-net or Van Cleve's bottom sampler, however, require even more time than utilization of our device.

Shadow and Noise Effects—Shading by the frame and net may bias daylight sampling, although the waters sampled in the upper estuarine areas of Texas are characteristically very turbid. Noise created by pounding during choppy seas may also tend to bias the results. These small problems, however, seem insignificant when compared to shadow and noise effects caused by pulling trawls and/or seines through the sampling area (*see* Hoese *et al.* 1968 for discussion).

Depth Limitations—The drop-net was used successfully in water depths ranging from 1 to 3.5 m. No data are available as to the maximum depth the net can be effectively used.

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THE INHIBITORY EFFECTS OF COPPER ON MARINE PHYTOPLANKTON[1]

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ABSTRACT

The inhibitory effect of copper ion on the growth of nine species of marine phytoplankton was examined in batch cultures under continuous illumination. The growth of three species of dinoflagellates and four species of diatoms was inhibited at 20 C in media containing 0.055 and 0.265 μg Cu/ml, respectively. The blue green alga *Coccochloris elabans* (strain 17a) failed to grow in the presence of 0.03 μg Cu/ml at 40 C, while the green alga *Dunaliella tertiolecta* Butcher survived copper concentrations near saturation values for seawater (≈ 0.6 μg Cu/ml) at 30 C.

Growth inhibition of *Skeletonema costatum* occurred at copper concentrations of 0.05 μg Cu/ml in light-dark cultures in which cell number and copper ion in the medium were maintained at constant levels by a single daily dilution and adjustment.

The initial copper uptake by the examined algae proceeded as an ion-exchange reaction, followed by a partial "desorption" of the copper taken up by the cells; this process reached equilibrium within 15 to 30 minutes at 25 C.

There was a negative correlation between the salinity of the media and the log of the ratio copper uptake—algal biomass. The relationship between the log of the same ratio and the temperature was fitted to a least squares regression; the slope of the regression yielded a Q_{10} value of 1.95 for the ratio.

[1] This work was supported by the Special Project Division of the Office of Saline Water, Department of the Interior, as part of the contract No. 14–01–0001–1161, to the Dow Chemical Company.

INTRODUCTION

The increasing occurrence of copper in coastal waters through the disposal of complex effluents has stimulated recent studies on both the uptake and physiological effects of this element on members of the marine biota (Pringle *et al.* 1968).

Phytoplankton concentrates copper from seawater by adsorption, allowing considerable amounts to be transferred to other trophic levels of the various food chains (Lowman *et al.* 1966). On the other hand, it has been established that the excretion of organic compounds by marine phytoplankton occurs under natural conditions (Hellebust 1965). Such substances as carboxylic acids, amino acids, carbohydrates and peptides, among others, can inactivate copper ion by chelation (Fogg and Westlake 1955). From the physiological point of view, copper has not been found to be a limiting factor to phytoplankton at concentrations found in natural environments. The concentration of this element in coastal and estuarine waters, however, has increased considerably as a consequence of human activities.

Marvin *et al.* (1961) reported that copper, in concentrations about ten-fold the normal for seawater (0.003–0.005 μg Cu/ml), was lethal to the red-tide forming dinoflagellate, *Gymnodinium breve.* In view of the possible different tolerance levels to copper among marine phytoplankton, modifications of the community structure may occur when relatively high copper concentrations are present in the marine environment. Changes in the quality and quantity of the species may have a great influence on the survival of other marine organisms (Loosanoff and Davis 1963).

Thus, the biological uptake of copper by plankton algae above the metabolic needs of the cells is of importance from a physiological, as well as an ecological, point of view. The primary purpose of this study was to obtain basic information on the inhibitory effects of relatively high copper concentrations on marine micro-algae, representing different algal groups, related to environmental parameters such as temperature and salinity.

MATERIALS AND METHODS

Organisms

Chlorophyta:	*Dunaliella tertiolecta* Butcher
Bacillariophyta:	*Skeletonema costatum, Cyclotella nana* (strain 13–1), *Thalassiosira fluviatilis, Nitzschia closterium*
Cyanophyta:	*Coccochloris elabans* (strain 17a)
Pyrrhophyta:	*Glenodinium foliaceum* Stein, *Glenodinium* sp., *Exuviaella* sp.

Culture Conditions

The cultures were grown at various temperatures, from 20 to 40 C, depending upon the organism, under a light intensity of 4000 lux from "daylight" fluorescent lamps.

The culture medium was local off-shore seawater, collected in one batch, en-

riched with nitrate, phosphate, silicate and vitamins as in medium "f" (Guillard and Ryther 1962). The amount of the trace element—chelating agent solution was reduced from 1 ml to 0.2 ml/l of medium.

The glassware was sterilized in an autoclave and the media sterilized by filtration through membrane filters. The observations were made with bacteria-free cultures; however, bacterial contamination was occasionally observed in some cultures.

Measurements

The static bioassay technique (ASTM 1964) was used to determine the inhibitory effect of copper on the cell division of the various algae. Seawater media containing final concentrations of copper in the range between 0.03 and 0.5 μg/ml were used for these tests. Copper inhibitory effects on *Skeletonema costatum* cultures grown in LD 15:9 light regimes were also investigated. These cultures were managed by a single daily dilution, after four hours in the light photoperiod, to give a standard cell concentration similar to that used in the static experiments. The copper concentration in the media was maintained at 0.05 ± 0.0025 μg/ml by daily adjustment. Growth rates were obtained from cell counts using a Neubauer Bright-line hemacytometer.

Experiments on the copper uptake by the cells were conducted in the following manner. A series of seawater solutions containing copper concentrations between 0.05 and 0.3 μg Cu/ml were prepared by adding copper as the chloride. Algal suspensions of the different cultures were added to these solutions in separate experiments to obtain a definite cell concentration, approximately the same used initially in the static bioassay tests. Blanks were simultaneously prepared by adding the same volume of membrane-filtered medium from the algal cultures to another set of seawater copper solutions. The length of the experiments was two hours. At different times aliquots were withdrawn from the flasks, filtered through Millipore HA, and the concentration of copper in the filtrates determined. The effects of time, temperature and salinity on the copper uptake by the algal cultures were determined by the same technique.

The analytical procedure for the analysis of copper was essentially that of Smith and McCurdy (1957), Gahler (1959) and Alexander and Corcoran (1967), with slight modifications. To 50 ml samples, 2 ml of a 10% hydroxylamine hydrochloride solution and 5 ml of a 0.1% ethyl alcohol solution of 2,9 dimethyl 1,10 phenanthroline (neocuproine) was added. The pH of the sample was adjusted to 5.5 with a sodium acetate solution and the orange complex extracted into 10 ml of chloroform. The extinction was measured in a 4 cm cell at 454 mμ in a Klett-Summerson Photoelectric Colorimeter. Duplicate samples were run in every case. The precision at the 100 μg Cu/l level was ± 2.5 μg Cu/l.

All the glassware used in these experiments was carefully washed in HCl solution, special detergents, and rinsed several times with de-ionized and glass-distilled water. The dry weight of the different algae was determined by filtering the algal suspensions through pre-rinsed, dried and tared Millipore filters HA, under reduced vacuum. The filtered cells were rinsed with distilled water and dried under vacuum at 40 C to constant weight.

RESULTS AND DISCUSSION

In the evaluation of a toxic compound and the physiological effects it produces on marine organisms, two factors are generally considered: the concentration of the toxic agent and the time of exposure. Since the time factor is of great importance in this type of test, it is common to report toxicity values for relatively brief periods of time; *e.g.*, 48 or 96 hours. The recorded physiological effect is the death of a percentage of the organisms assayed, TLm = "Median tolerance limit" (Doudoroff 1951). With algae, the physiological measurement is based largely on the reduction of their photosynthetic or cell division rates. This criteria, however, is rather difficult to apply to algae because at certain toxicant concentrations algal cultures may show an extended lag phase before cell division starts.

Another factor to be considered is that part of the toxicants, particularly heavy metals, are removed from the medium by the cells. On the other hand, certain species can be adapted to relatively high concentrations of toxicants and in some cases resistant types may develop (Kellner 1955).

Maloney and Palmer (1956) reported on the toxicity of copper to thirty freshwater algal cultures, concluding that copper had a selective toxicity, rather than a general effect, on the algae at concentrations between 0.05 and 0.5 μg Cu/ml.

In the present study the toxicity of copper to nine species of marine phytoplankton was determined in a series of experiments in batch cultures under continuous illumination; the results are shown in Table 1. Dinoflagellates were more sensitive to relatively low copper concentrations than diatoms. The most resistant organism was the green alga, *Dunaliella tertiolecta* Butcher. This alga was capable of growing in media containing copper concentrations near saturation values for seawater ($\approx$0.6 μg Cu/ml).

It should be pointed out that the results of these experiments give only a relative measure of the inhibitory effects of copper on marine micro-algae.

Additional experiments on light-dark cultures of *Skeletonema costatum*, in

TABLE 1

Inhibitory copper levels and concentration factors for nine species of marine phytoplankton

Algae	Initial cell number cells/ml	Temp. C	Illumination[1] 4000 lux	Inhibitory copper[2] concentrations μg/ml	C. F.[3] at 30 C
Skeletonema costatum	3×10^5	18	LD 15:9	0.05	....
Cyclotella nana (13–1)	2×10^5	20	LL	0.230	....
Nitzschia closterium	6×10^4	20–30	LL	0.190–0.130	....
Skeletonema costatum	3×10^5	20–30	LL	0.250–0.160	....
Thalassiosira fluviatilis	3×10^4	20–30	LL	0.265–0.180	850:1
Glenodinium sp.	9×10^4	20–30	LL	0.055–0.03	....
Glenodinium foliaceum Stein	6×10^3	20–30	LL	0.055–0.03	1500:1
Exuviaella sp.	2×10^4	20–30	LL	0.045–0.025	....
Dunaliella tertiolecta Butcher	5×10^4	35	LL	>0.5	600:1
Coccochloris elabans (17a)	$\approx 1 \times 10^6$	40	LL	0.03	1100:1

[1] Continuous light is designated by LL and a light-dark cycle by LD.
[2] Initial copper concentration in the medium before the addition of the inocula.
[3] Concentration factors—Calculated by wet weight of cells, arbitrary dry weight $\times$ 10.

which the cell number and the copper concentration were maintained at constant levels, showed that cell division was inhibited in media containing 0.05 μg Cu/ml at 18 C. The concentration of copper in this experiment was only one-fifth of the concentration that inhibited the growth of *Skeletonema costatum* in batch cultures under continuous illumination at 20 C.

When algal cultures are exposed to heavy metals in solution at concentrations above the metabolic needs of the cells, two main processes generally take place; binding the ions to the surface of the cells and interference with the metabolic functions.

Cellular material consists of macromolecules containing carboxyl, phosphate, amino or sulfhydryl groups, which are reactive sites on enzymes. The more electronegative metals, notably copper and mercury, are strongly bound to these groups. Thus, their inhibitory effects on living cells tends to be non-specific.

Copper, as in the case of mercury and iodoacetate, can inactivate enzymes related to the respiratory metabolism; consequently, endogenous respiration can be severely impaired. Hochachka and Teal (1964) reported that iodoacetate, arsenite and malonate, among other compounds, inhibited the endogenous rate of respiration in the dinoflagellate, *Gymnodinium nelsonii*. Moreover, cells growing in light-dark cycles were more sensitive to some of these compounds in the middle of the light-period and at the beginning of the dark-period. Hassall (1962, 1963), on the other hand, indicated that the specific effect of copper on the respiration of *Chlorella vulgaris* was dependent on the environmental conditions.

Because accumulation is so essential in considering the toxic effects of copper on algae, a study was made on the nature of the copper uptake by the cells. The method used for these experiments, however, does not differentiate between the extracellular and intracellular location of the copper in the cells.

In all the short-term copper uptake experiments on species of the different algal groups, there was an instantaneous uptake followed by a partial release of the copper by the cells, with equilibrium being reached within 15 to 30 minutes at 25 C.

A similarity in the behavior of the algae was determined in experiments carried out at toxic copper levels (Figs. 1 and 2), as well as sub-lethal concentrations (Fig. 3). The cultures exposed to sub-lethal levels resumed growth after a lag period that was copper concentration dependent.

It is not clear whether the copper released by the cells at sub-lethal levels is a consequence of a change in the permeability of the cells with the loss of cellular constituents or an increased excretory activity of the cells, acting as a protective mechanism of detoxication. A similar effect, but caused by a light intensity stress on marine phytoplankton, was reported by Hellebust (1965). The breakdown on the cell wall permeability must also be considered at lethal levels (Rothstein 1959).

Among the excretory products of microalgae, amino acids and peptides can inactivate copper ions by chelation, considerably reducing the ionic copper concentration in the medium. These copper chelates, however, are compounds with very low stability constants (Johnston 1964) as compared to the organic complexing agents used in the analytical techniques for copper determination.

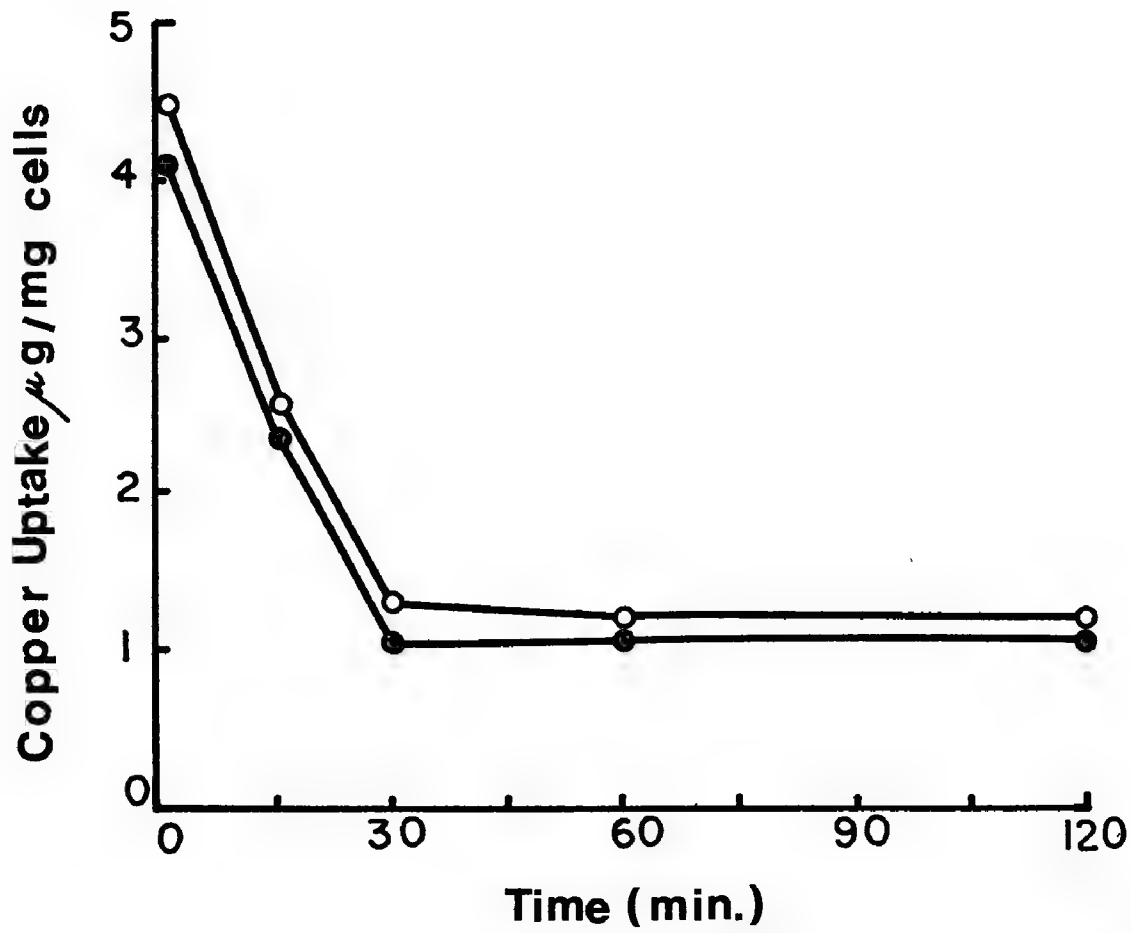

FIG. 1. Influence of time on the copper uptake by ○ *Thalassiosira fluviatilis* and ● *Skeletonema costatum*, initial copper concentration 0.210 μg/ml, temperature 25 C. Each point is the average of three sets of experiments.

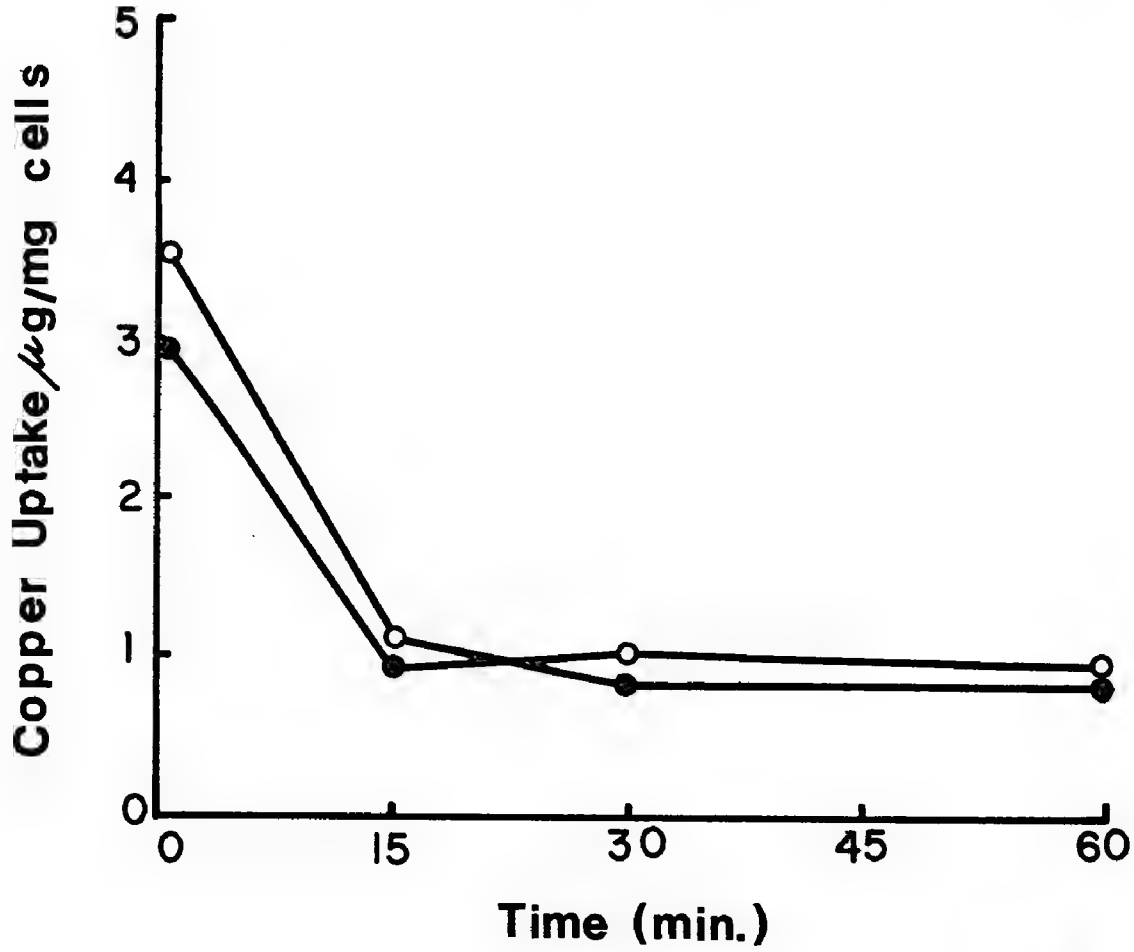

FIG. 2. Influence of time on the copper uptake by ○ *Glenodinium* sp. and ● *Glenodinium foliaceum*, initial copper concentration 0.110 μg/ml, temperature 25 C.

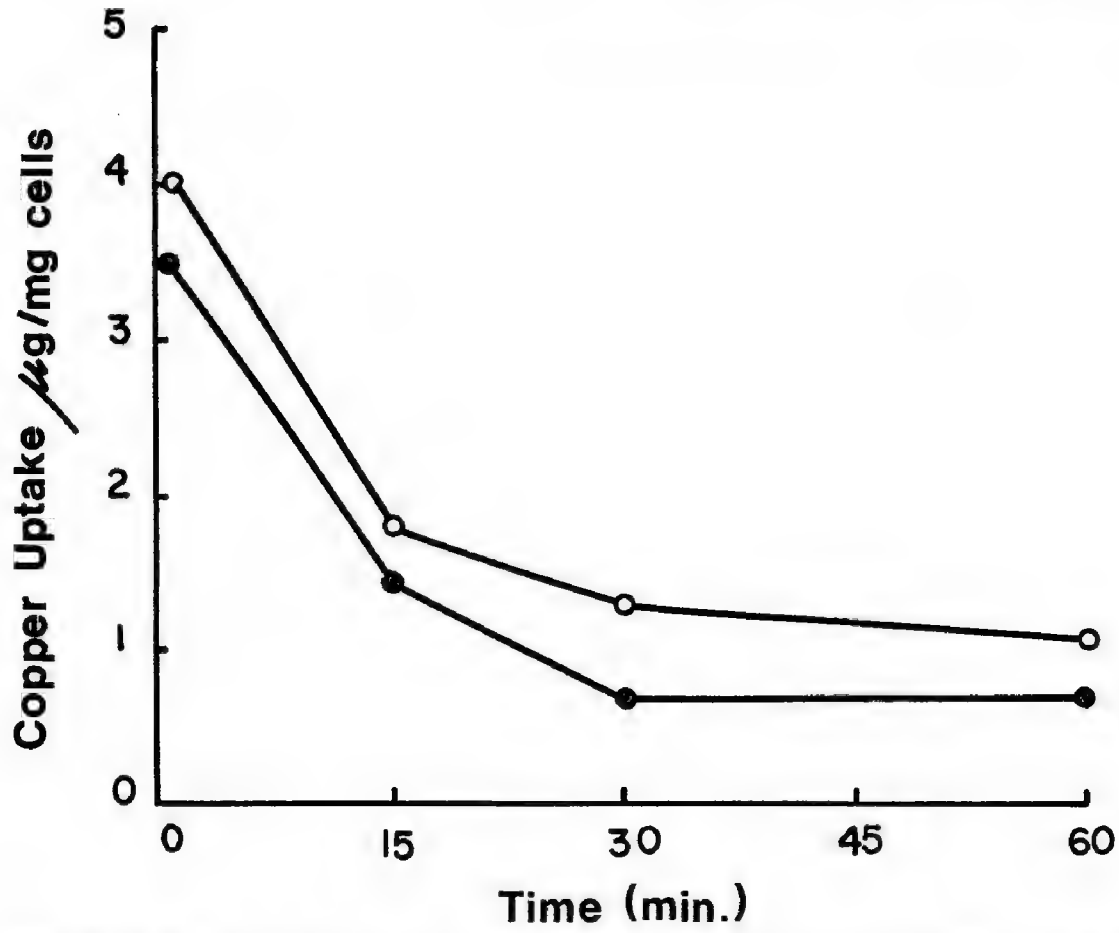

FIG. 3. Influence of time on the copper uptake by ○ *Thalassiosira fluviatilis* and ● *Dunaliella tertiolecta*, initial copper concentrations 0.152 and 0.174 µg/ml, respectively, temperature 25 C.

The results of the experiments on the effects of the initial copper concentration on the uptake indicated that the copper retained by the cells was proportional to the external concentration in the medium (Fig. 4).

The empirical Freundlich isotherm has been widely used to express non-metabolic adsorption in many different systems. The mathematic relationship is given by:

$$\frac{x}{m} = kc^n, \tag{1}$$

where x is the amount of solute taken up from the solution, c is the concentration of the solute in solution, m the mass of adsorbent, and k and n are constants for a particular system at a constant temperature. This expression has found previous application in limnology, describing the uptake of copper by plankton (Riley 1939) and the uptake of various ions by *Chlorella* cultures (Knauss and Porter 1954).

According to the results of the experiments herein reported, the adsorption isotherm equation can not be applied to the copper uptake by the investigated algae.

Enrichment factors calculated from the short-term copper uptake experiments varied considerably between the different classes of algae; they were found to be inversely related to the inhibitory copper concentrations determined by the static toxicity bioassay (Table 1). Thus, the growth of organisms with high copper uptake per unit of algal biomass (*i.e.*, dinoflagellates) was inhibited at lower copper concentrations.

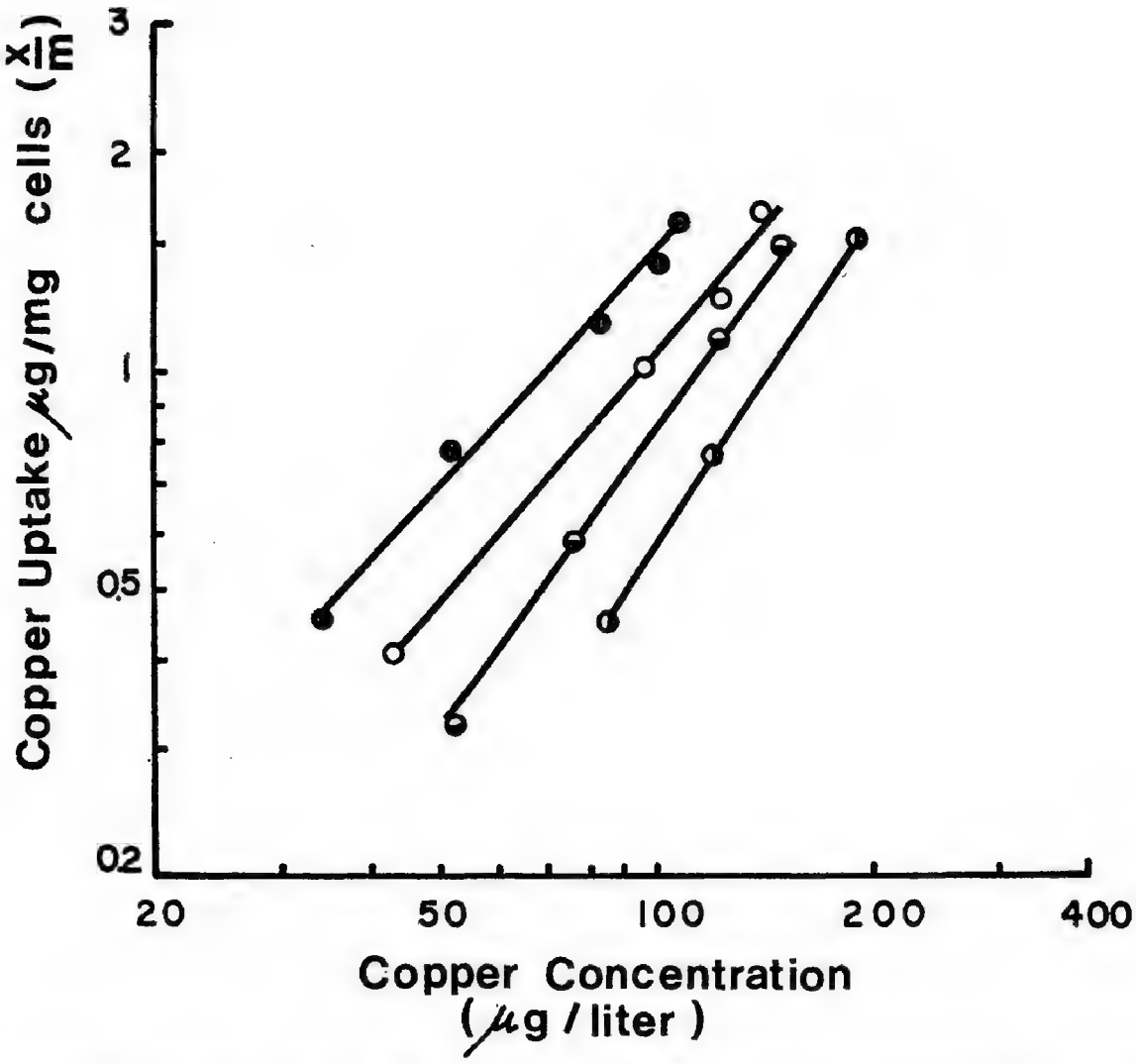

FIG. 4. Copper uptake (µg/mg cells, dry weight) by ● *Glenodinium foliaceum*, ○ *Coccochloris elabans* (strain 17a), ◒ *Thalassiosira fluviatilis* and ◑ *Dunaliella tertiolecta*, as a function of the copper concentration in solution, temperature 30 C.

The copper uptake by the algae also showed a definite temperature-dependence within the studied experimental range, 20 to 35 C. The relationship between the log copper uptake by the cells and the temperature (Fig. 5) was fitted to a least squares regression equation:

$$\log \text{copper uptake } (\mu g/mg) = 0.805 + 0.029\ C. \qquad (2)$$

The slope of the regression yielded a Q_{10} value of 1.95 for the copper uptake; the 95% confidence limits fell between 1.69 and 2.26.

Several experiments were also conducted to ascertain the effects of salinity on the copper uptake by three species of diatoms. In every case, increased salinities reduced the copper uptake (Fig. 6). The relationship between the log copper uptake by the cells and the salinity was also fitted to a least square regression equation:

$$\log \text{copper uptake } (\mu g/mg) = 0.393 - 0.011 \text{ ppt.} \qquad (3)$$

SUMMARY

The response of marine phytoplankton to copper ions above the normal levels found in the marine environment varied according to the species. Copper had a selective toxicity, rather than a general effect, at concentrations between 0.03 and 0.5 µg Cu/ml. Dinoflagellates were more sensitive to copper than diatoms,

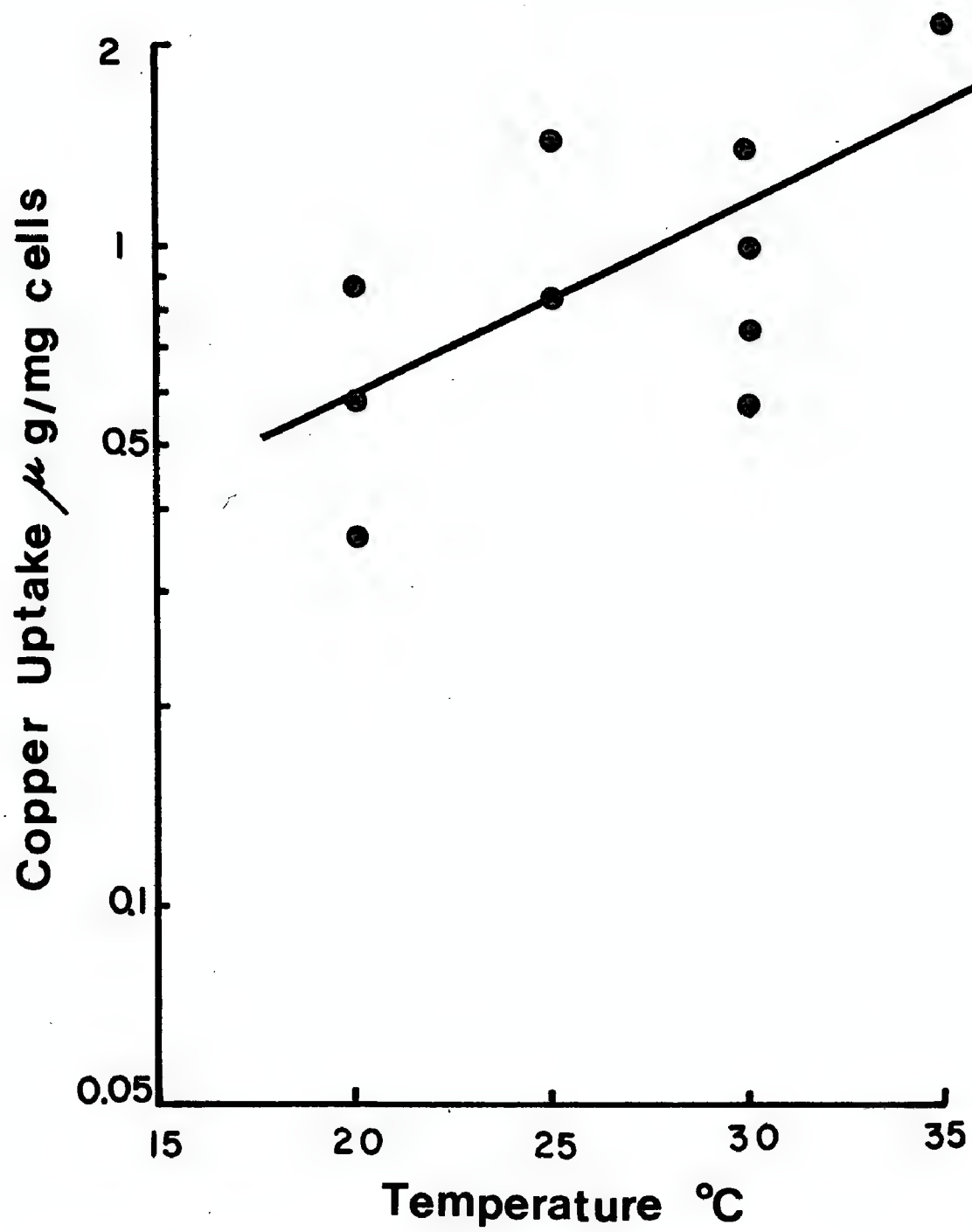

FIG. 5. The effect of temperature on the copper uptake (μg/mg cell, dry weight) by *Skeletonema costatum, Thalassiosira fluviatilis, Glenodinium foliaceum, Dunaliella tertiolecta* and *Coccochloris elabans,* salinity 31.75 ppt.

while one species of green alga survived at copper concentrations near saturation values for seawater.

Short-term copper uptake experiments on species from different algal groups indicated that concentration factors were inversely related to the inhibitory copper concentration in the medium. The copper uptake by the cells at lethal and sub-lethal levels consisted of an instantaneous ion-exchange type of reaction, followed by partial desorption of the copper taken up. It was suggested that the release of the copper by the cells occurred through an increased excretion of organic compounds as a mechanism to reduce the stress. A passive permeation of the copper through the cells, at lethal levels, must also be considered.

There was a positive correlation between the log copper uptake—algal biomass and the temperature and a negative correlation for the same ratio regarding the

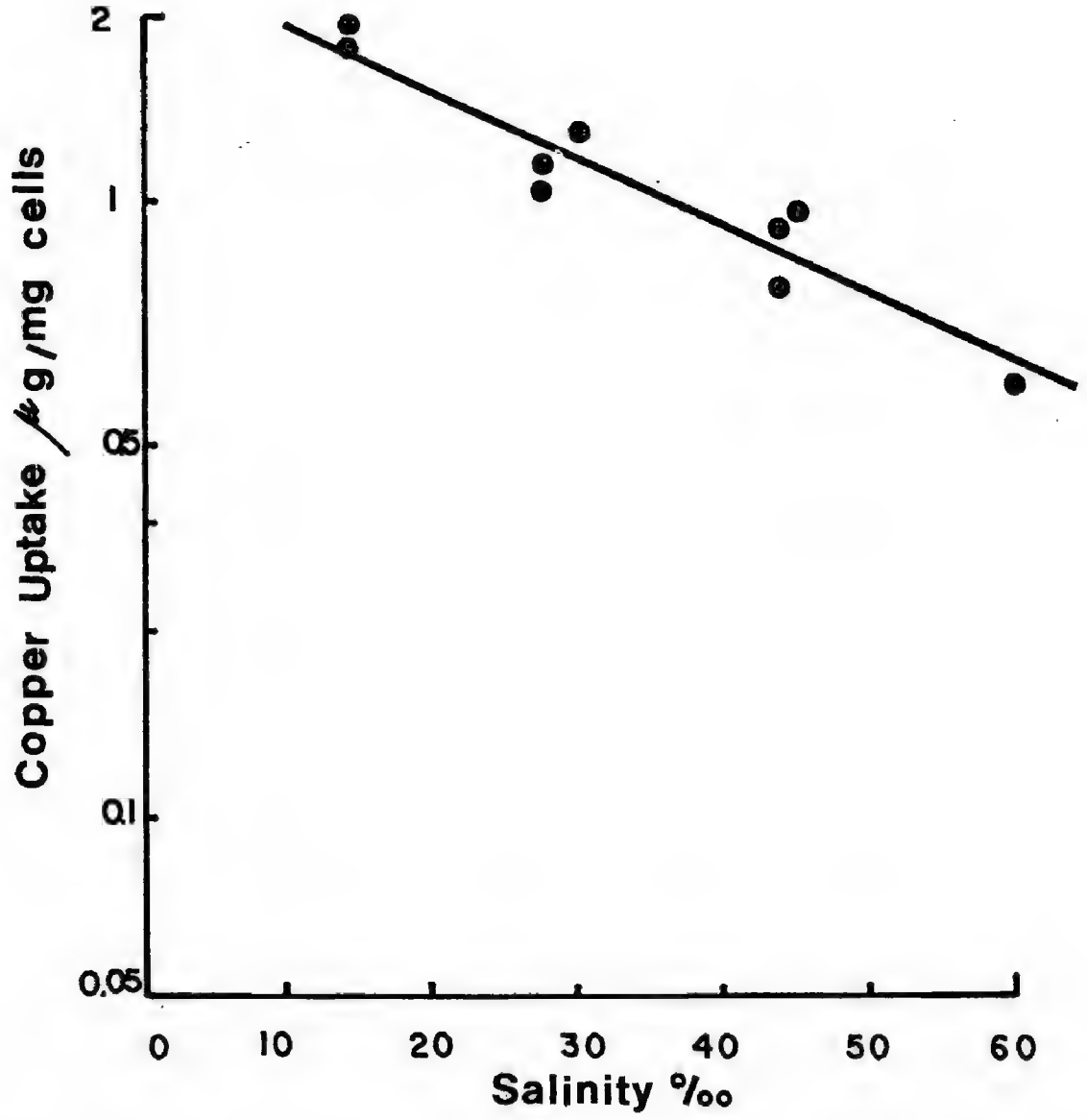

FIG. 6. The effect of salinity on the copper uptake (µg/mg cells, dry weight) by *Skeletonema costatum*, *Nitzschia closterium* and *Thalassiosira fluviatilis*, temperature 25 C, initial copper concentration 0.150 µg/ml.

salinity parameter. An increase of 10 C within the tolerance temperature range for the different species reduced the copper concentration that was lethal to the algae.

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FIELD AND CULTURAL STUDIES ON THE SEASONAL PERIODICITY OF GROWTH AND REPRODUCTION OF SELECTED TEXAS BENTHIC MARINE ALGAE[1]

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ABSTRACT

Seasonal observations in nature in the Port Aransas, Texas area in the Gulf of Mexico have been combined with cultural studies in the laboratory in an attempt to elucidate the causal factors of the distinctive seasonal alteration of the benthic marine algal flora of the region.

Monthly field studies, in which the seasonal distribution of species was determined, were conducted over a period of 24 months. Correlations were sought between changes in the algal vegetation and variations of environmental factors. However, it was not possible to correlate changes in the algal flora with variations of single parameters, since many factors vary simultaneously in nature.

An attempt was made to simulate the natural environment in the laboratory so that changes of the environment could be experimentally controlled and varied at will. A series of incubators, and a cross light intensity and temperature gradient culture apparatus were employed.

The following 12 brown and red algae were cultured in varying light intensity, temperature and daylength regimes and the effects of these factors on their growth and reproduction were ascertained: *Ectocarpus siliculosus, Giffordia mitchelliae, Petalonia fascia, Erythrocladia sub-*

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integra, Goniotrichum alsidii, Bangia fuscopurpurea, Porphyra leucosticta, Acrochaetium crassipes, A. flexuosum, Ceramium strictum, Centroceras clavulatum and *Polysiphonia boldii.*

The data obtained from the cultural studies were used in an attempt to explain the observed seasonal alteration of the flora in nature. The results support the widely held view that temperature is of primary importance in the seasonal distribution of algae in nature. Seasonal variations in daylength, acting alone or in conjunction with temperature variations, have in addition been implicated as a causal factor in the seasonal alteration of the flora. Seasonal variations in light intensity, however, do not appear to be involved.

INTRODUCTION

Studies of the benthic marine algae of the mid-Atlantic North American coast from Cape Cod to Cape Kennedy, and the northern Gulf of Mexico, reveal pronounced seasonal changes in growth of the algae. Certain algae undergo maximum growth during the summer or during the winter and early spring, and decline in growth or even disappear during the unfavorable portion of the year; these are often referred to as summer and winter annuals, respectively. Others grow throughout the year in certain areas and are referred to as perennials.

In an attempt to explain the seasonal alteration of the flora of a given area, investigators have sought to correlate the observed changes in the algal vegetation with changes in environmental factors in nature.

Hoyt (1920), in his classic study, observed distinct seasonal changes in the algal vegetation of North Carolina and concluded that the flora comprised "northern and southern elements, with the latter predominating." He correlated the disappearance of the summer flora in October and November and the subsequent appearance of the winter-spring flora with the greatest decrease in water temperature; the appearance of the summer flora in April and the disappearance of the winter flora in May were attributed to a rise in the temperature of the water.

Williams (1948, 1949) conducted a seasonal ecological survey of the marine algae of a jetty at Cape Lookout and confirmed Hoyt's conclusions concerning the general nature of the North Carolina flora. He considered that "the dual nature of the algal vegetation may be attributed mostly to the wide annual range of temperature." The water temperatures are high enough to support a permanent flora of tropical affinity, which is augmented by an annual winter-spring flora with northern affinities and an annual summer-fall flora with tropical affinities.

A seasonal survey of the algal vegetation of lower Chesapeake Bay was conducted by Zaneveld and Barnes (1965). They also reported climaxes of vegetative activity in winter and summer, followed by a decline or disappearance of certain species. The changes in the vegetation were considered to be solely the result of seasonal temperature fluctuations.

Seasonal studies of the periodicity of algal vegetation in northern Florida have been conducted by two investigators. Humm (1952) studied the algae on the rocks at Marineland, on the Atlantic coast, without proffering a hypothesis for the observed seasonal changes in the algae. He concluded that the flora is composed of predominantly tropical species which do not disappear during the winter, although several become reduced in abundance and stature. Earle (1969) studied the brown algae of the eastern Gulf of Mexico and indicated that at least 13

species of brown algae, including *Ectocarpus siliculosus* and *Petalonia fascia*, occur only in the winter and spring, while several Dictyotales of tropical affinity are present only during the summer in the northern part of the Gulf of Mexico. She attributed the differences in seasonal occurrence to changes in the water temperature, which is 28–30 C in summer and 13–15 C during the winter.

The most detailed comparisons between observed seasonal changes in algal vegetation and environmental factors measured in the field were conducted by Conover (1958, 1964). The first study was carried out in Great Pond, an estuary on the southern shores of Cape Cod. He discerned two major sequences in the seasonal vegetation: a large summer one, including *Agardhiella tenera* and *Hypnea musciformis;* and a smaller winter-spring sequence including *Ectocarpus siliculosus, Petalonia fascia, Bangia fuscopurpurea,* and *Porphyra leucosticta.* He observed that the maxima and minima of the two seasonal portions of the flora were associated with the maxima and minima in temperature and insolation, and suggested that these two factors play the leading role in the seasonal growth of the algae in Great Pond. He considered the winter-spring species to be adjusted for optimum net photosynthesis at low temperatures and illumination. The summer flora he considered to be composed of two groups of species: the first attaining maximum growth rates during the illumination maximum during June and early July, and possibly adapted for optimum net photosynthesis at maximal light and submaximal temperature; the second group attained maximum growth during the temperature maximum in August and was possibly adapted for optimum net photosynthesis at maximal temperature and submaximal light. The second study concerned the seasonal periodicity of occurrence of marine benthic plants from the lagoons and jetties of the Gulf coast of Texas. He again found a large group of species of summer appearance (*e.g., Laurencia poitei* and *Digenia simplex*) and a smaller group of winter-spring occurrence (*e.g., Petalonia fascia, Bangia fuscopurpurea,* and *Porphyra leucosticta*). However, he observed that the maximum growth period of the summer flora, which occurs in May, June and early July, appeared to be correlated with the illumination rather than the thermal maximum which occurs in August, although possibly the high water temperatures of late summer contributed to the decline of the vegetation at that time by producing high metabolic rates which limit growth.

The final analysis of the seasonal distribution of algae must depend on cultural studies. Several factors vary simultaneously in nature, so that it is generally not possible to correlate the observed seasonal changes in the algal vegetation with variations of single parameters in the field. Attempts must be made to simulate the natural environment in the laboratory, so that variations of each parameter may be experimentally controlled. The effects of each factor on algal growth and reproduction may then be ascertained.

Seasonal observations in nature in the Port Aransas, Texas area have been combined with cultural studies in the laboratory in the present study in an attempt to further our understanding of the causal factors of the seasonal periodicity of growth and reproduction of benthic marine algae. Monthly field observations were conducted on the algal flora in the vicinity of Port Aransas. Environmental data were compiled, and correlations were sought between the observed seasonal

alteration of the algal vegetation and changes in several environmental factors. Hypotheses similar to those established by previous investigators were established as possible causes of the seasonal changes of the algal flora. The hypotheses were subsequently tested in culture by the use of controlled experiments, in which the various environmental parameters could be varied independently and their effects on the algae separated.

MATERIALS AND METHODS

Field Studies

Monthly observations were conducted over a 2-year period from June 1967 to May 1969 on the benthic marine algae of the Port Aransas area. Observations were made on the Port Aransas jetties and in Aransas and Redfish Bays (Fig. 1). A list of the 12 algae that were studied in culture, with a brief consideration of growth and reproduction, is presented below.

Phaeophycophyta

Ectocarpus siliculosus (Dillwyn) Lyngbye occurred in the relatively protected shallow waters of Aransas Bay from December to April and was apparently absent for the remainder of the year. Plurilocular organs were the only type of reproductive body observed.

Giffordia mitchelliae (Harvey) Hamel was collected on the jetty at Port Aransas annd in Aransas Bay. This species has been found in most months of the year, but its seasonal distribution was erratic, and it was often apparently absent. Plurilocular organs were the only type of reproductive body observed.

Petalonia fascia (O. F. Mueller) Kuntze was present on the jetty at Port Aransas from December to April and was apparently absent during the remainder of the year. Plurilocular organs were the only type of reproductive body observed.

Rhodophycophyta

Bangiophycidae

Erythrocladia subintegra Rosenvinge was present as an epiphyte on a variety of larger algae throughout the year. It was collected almost exclusively at Port Aransas. Reproduction was by monospores.

Goniotrichum alsidii (Zanardini) Howe was present as an epiphyte on a variety of larger algae from June to December at Port Aransas and in Aransas and Redfish Bays. The thallus was invariably uniseriate and the pluriseriate condition was observed on only one occasion. Reproduction was by monospores.

Bangia fuscopurpurea (Dillwyn) Lyngbye was collected on the jetties during winter and early spring, but was found in small amounts on one occasion as early as October and as late as July. Alpha and β spores were commonly observed. *Bangia* was apparently absent for the rest of the year. The putative alternate phase, *Conchocelis*, was not observed in the field.

Porphyra leucosticta Thuret occurred on the jetties from January to April; α

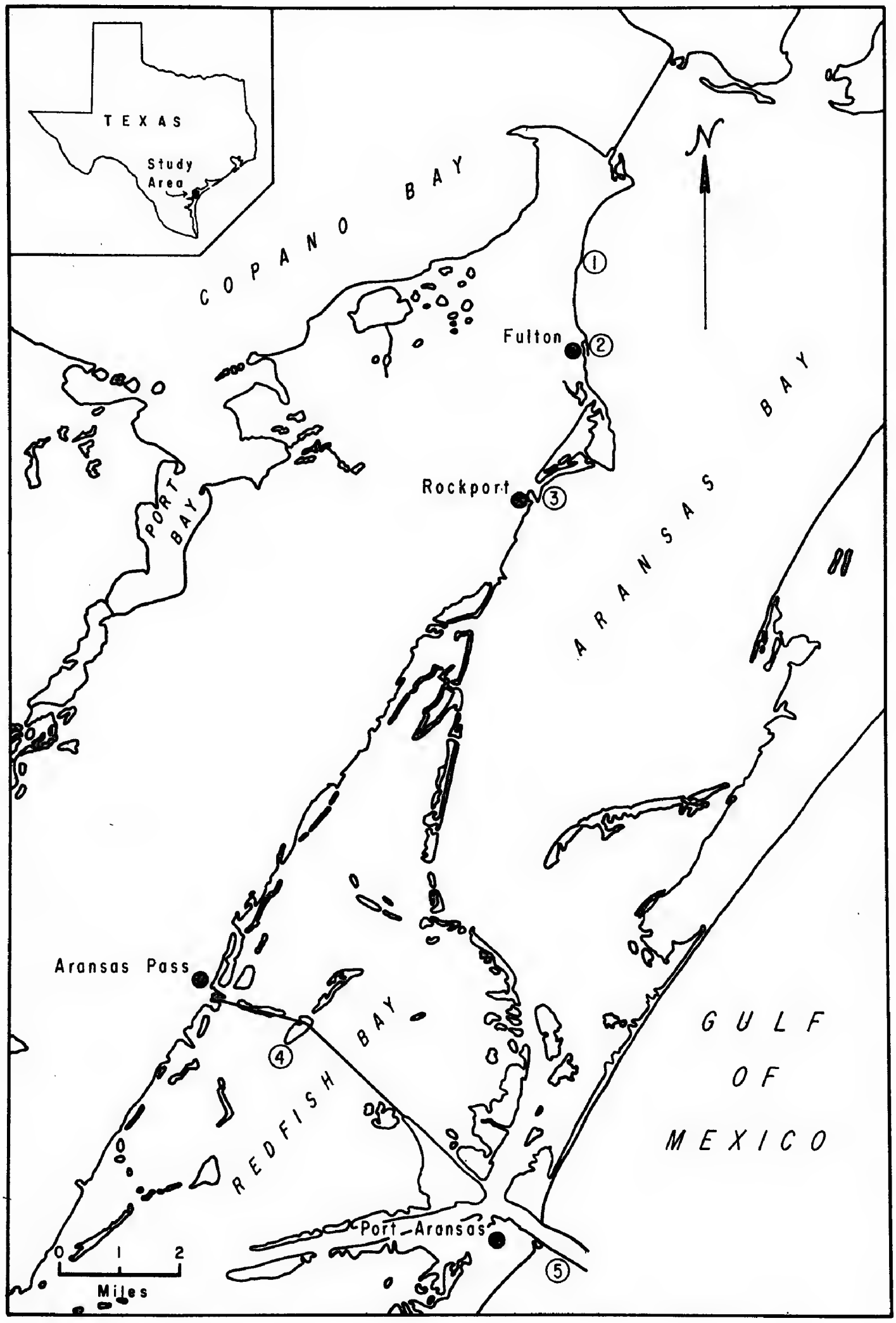


FIG. 1. Location of the 5 stations at which monthly collections of algae were made. Redrawn from USC & GS map 1285.

and β spores were invariably observed. The alga was apparently absent during the summer and the alternate phase, *Conchocelis*, was not collected in nature.

Florideophycidae

Acrochaetium crassipes Boergesen was observed throughout the year as an epiphyte on a great variety of larger algae, mainly at Port Aransas, and also in Aransas Bay on two occasions. Reproduction was solely by monospores.

Acrochaetium flexuosum Vickers occurred throughout the year as an epiphyte on a great variety of larger algae at Port Aransas and in Aransas Bay. Reproduction was by monospores.

Ceramium strictum (Kuetzing) Harvey occurred at Port Aransas with maximum growth in summer. Tetrasporangia were the only type of reproductive body observed.

Centroceras clavulatum (C. Agardh) Montagne was found at Port Aransas, where it demonstrated maximum growth during the summer. Minute specimens of the alga were occasionally found in winter and spring. Tetrasporangia were the only type of reproductive body observed.

Polysiphonia boldii (Wynne and Edwards manuscript) grew and reproduced throughout the year but was apparently more common during the summer months. It was found at Port Aransas and in Aransas and Redfish Bays.

Cultural Studies

Twelve brown and red algae from the Port Aransas region were cultured under varying conditions in an attempt to elucidate the role of the various parameters in determining seasonal changes in the growth and reproduction of the algae.

Selection of Cultural Parameters and Their Measurement

Light

Pronounced seasonal fluctuations in the intensity of light take place. Brownsville, 150 miles from the study area, has an average monthly maximum in June of 645 langleys per day, and an average monthly minimum in December of 256 langleys per day (Fig. 2). Light intensity variations may possibly play a major role in the seasonal changes of algal vegetation.

T 12 daylight fluorescent lamps were used as the light source throughout the study. This type of lamp was selected since its emission spectrum is closer to the absorption spectra of whole-cell homogenates of red and brown algae, as determined by the method of Shibata (1958), than those of other types of fluorescent lamps. The algae maintain a typical morphology, and grow and usually reproduce in this artificial source of light, although it constitutes only an approximate simulation of natural light. The light quality was not varied.

Light intensities were determined with a Weston illumination meter model 756 fitted with a quartz filter. The foot-candle measurement of light intensity obtained by the illumination meter is only an approximate measurement of the

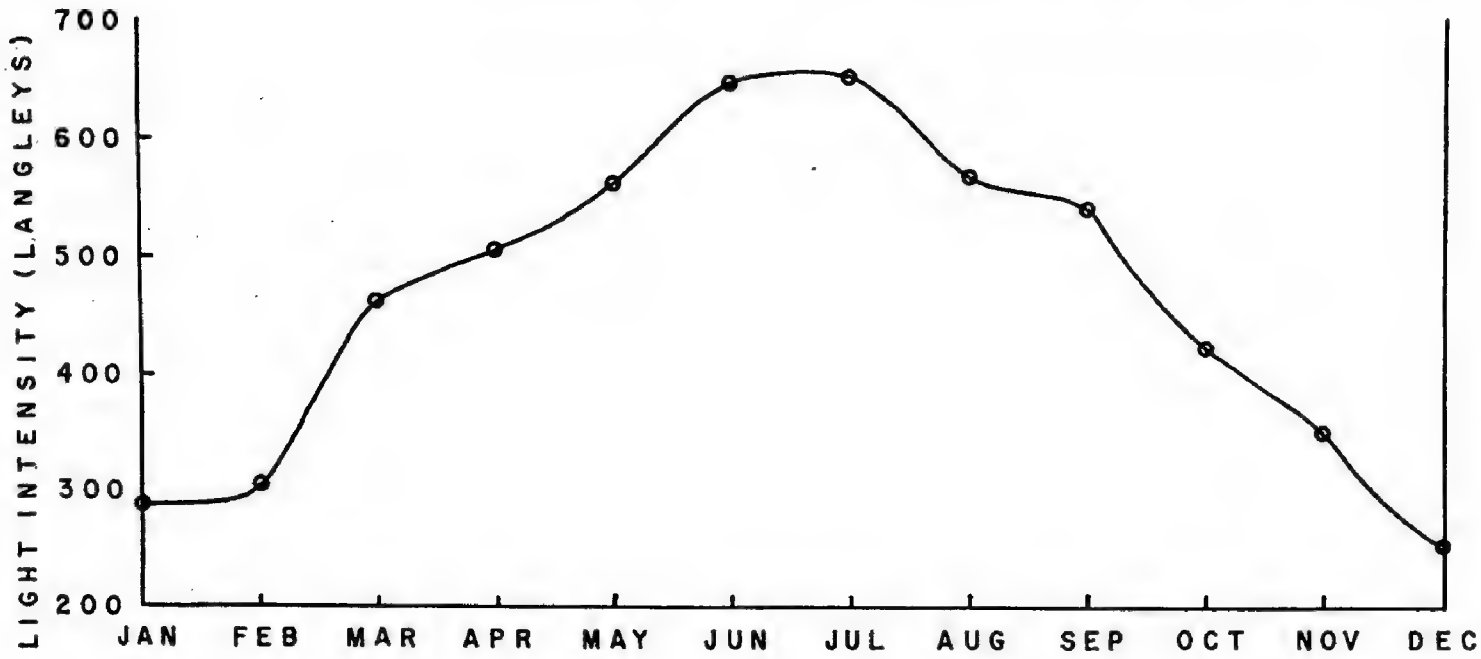


FIG. 2. Solar radiation at Brownsville, Texas. Average monthly values 1965–1967. Drawn from data obtained from U.S. Dept. Comm. Weather Bureau. Climatological data. National summary: 1965–67.

total light energy emitted by the source, since it is based on the standard human eye sensitivity curve which peaks at about 550 mμ. To obtain more meaningful light energy values, the illumination meter was calibrated against a Standard Lamp from the National Bureau of Standards. The following conversion factor was obtained: 1 ft c = 33.0 ergs/cm²-sec.

It was not possible to obtain light intensities as high as those occurring in nature with the equipment available, so that seasonal light variations in nature could not be exactly duplicated. A range of intensities up to the maximum attainable value in the laboratory, approximately 700 ft c, was employed.

Light intensities were measured at the start and conclusion of each experiment since the energy of the light source decreased with time. The values are expressed as the average, with the total range.

Temperature

A large seasonal variation in the surface water temperature takes place in the study area. The minimum average monthly temperature in January is 12.7 C, and the maximum average monthly temperature in July is 30.0 C, giving a seasonal range of 17.3 C (Fig. 3). Temperature may thus be expected to play a major part in determining the seasonal changes of algal vegetation in the area.

The range of temperature used in the study corresponded in a general way to the maximum and minimum mean monthly surface water temperatures at Port Aransas.

The temperatures of the medium in the Petri dishes in the incubators were determined by centigrade thermometers placed in the incubators, since it was assumed that the temperatures of the medium in the dishes were equilibrated with the air temperature inside the incubators. The temperatures of the medium in the Petri dishes in the cross-gradient culture apparatus were determined directly with a thermistor.

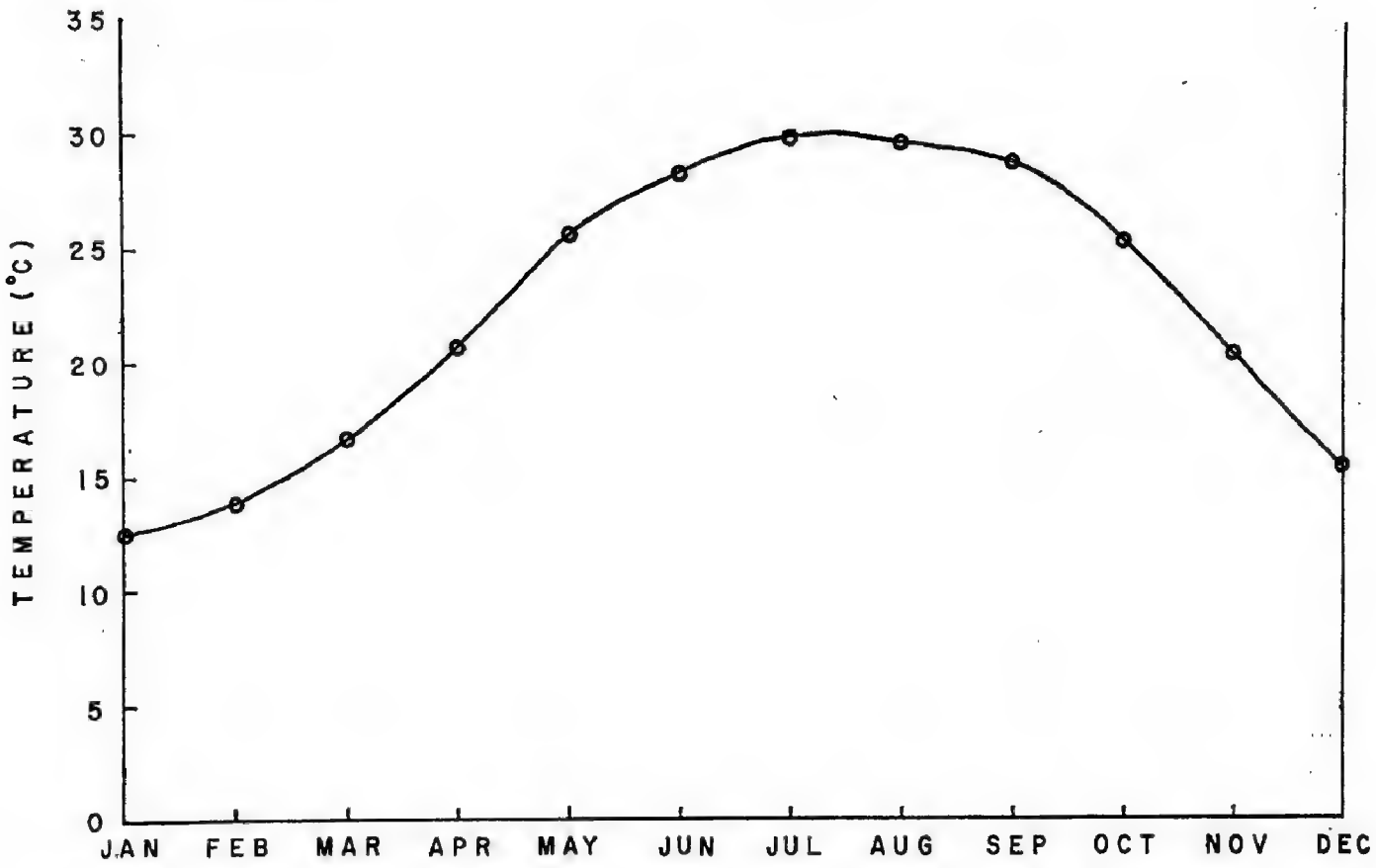


FIG. 3. Mean monthly surface temperatures for Port Aransas, Texas. Drawn from data obtained from U.S. Dept. Comm. Surface water temperature and salinity, Atlantic coast, North and South America, 1965.

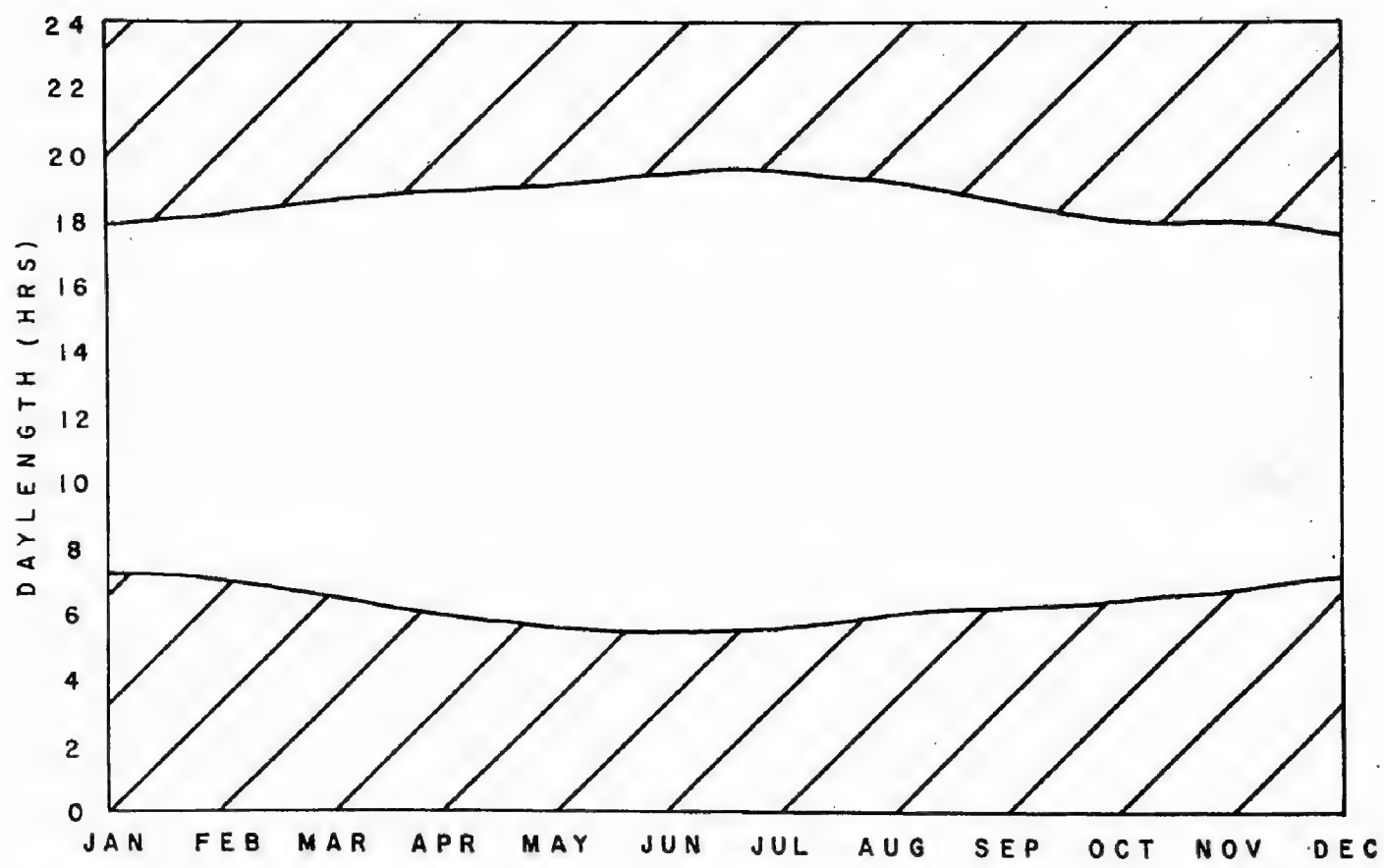


FIG. 4. Average monthly daylength values at Port Aransas, Texas. Drawn from data obtained from Table of Sunrise and Sunset at Corpus Christi, Texas. No. 1279 U.S. Govt. Printing Office, Washington, 1965.

Daylength

The maximum average monthly daylength is 13-hr 54 min in June and the minimum average monthly daylength is 10-hr 24 min in December at Port Aransas, Texas at a latitude of approximately 27°N (Fig. 4). Possible effects of varying daylengths on the seasonal growth and reproduction of the algae were investigated because of reports in the literature of profound effects of daylength variations on marine algae.

Nutrition

Seasonal variations in the concentrations of nutrients in the sea may play a determining role in the seasonal growth and reproduction of algae, but possible effects of nutrient variations were not investigated during the current study.

A medium was required which could support good growth for a large number of algae from different taxonomic assemblages. Von Stosch's enriched natural seawater medium as cited by Ott (1966) satisfied this criterion and was employed throughout the study.

The seawater was collected in the surf at Port Aransas and generally used within one month of collection. It was filtered through Whatman's No. 1 filter paper and sterilized in 5-liter lots by autoclaving for 30 min prior to the addition of the enrichment.

The algae were cultured in 60 × 15 mm pyrex Petri dishes containing 20 ml of medium.

Natural seawater media have the disadvantage of having an unknown chemical composition, which may vary with the sample. This is especially true where irregular heavy rains, with subsequent increased runoff, wash terrestrial material into the sea, as in the Port Aransas area. Artificial sea water media with defined chemical compositions were originally developed for the growth of unicellular algae, and a single medium generally will not support healthy, prolonged growth of a wide range of more nutritionally exacting multicellular algae (Provasoli 1963).

All experiments were conducted in unialgal culture, in which fungi and bacteria were presumably present. Two effects may be responsible for the different growth rates or reproductive states of a culture under varying environmental conditions. Firstly, the direct response of the alga itself to the parameter or, secondly, the response of the non-algal population in the culture which may indirectly influence the growth rate or reproductive state of the alga. Fries (1966) provided evidence for the latter when she demonstrated optimal growth temperatures in pure culture much higher than the temperature for the warmest month of the year in nature for *Goniotrichum elegans*, *Nemalion multifidum* and *Polyisphonia urceolata*.

Salinity

The effects of salinity variations on growth and reproduction of the algae were not investigated because regular seasonal changes in the algal vegetation occurred in spite of irregular salinity fluctuations in nature in the Port Aransas area.

The salinity of the seawater used in the culture experiments was determined

with a Goldberg Refractometer (American Optical Co., Model 10402), as outlined by Behrens (1965). The salinity was adjusted to 28–32 ppt by vacuum distillation or by dilution with distilled water, if the salinity was not so on collection.

Evaporation of the medium, with a concomitant increase in salinity, took place during the course of each experiment. Percentage increases in the salinity of the medium after one week, over the temperature range used in the study, were as follows (determined from percentage decreases in the volume of the medium in the Petri dishes; each value is an average of six readings): 0.6% at 10.5 C, 0.6% at 14.5 C, 2.9% at 19 C, 5.6% at 22.5 C, 8.6% at 25.5 C and 11.8% at 28.5 C.

Apparatus Employed

Two sets of apparatus were used for the investigation. The first consisted of a series of five General Electric incubators, Model 805, each fitted with a battery of three lamps mounted on a board and suspended from the top of the incubation chamber. The light intensity was varied by varying the number of lamps used and by adjusting the distance between the light source and the Petri dishes. The lamps were cycled by an interval timer. The incubator temperatures given represent day temperatures. The temperature fell by about 4 C within 2 hr when the lights went off and took about the same time to regain the daytime value when the lights came on again. The second apparatus comprised a cross light-intensity and temperature gradient culture apparatus, modified after the principle of Halldal and French (1958), by Edwards and Van Baalen (in press), to permit the culture of benthic marine algae under many combinations of light intensity and temperature under a given daylength.

Experiments Conducted

A total of five experiments were conducted in the incubators. The first three experiments investigated the effects of light intensity, daylength and temperature independently of each other. In the light intensity and temperature experiments, the parameter under investigation was varied, while the remaining two factors were held constant. In the daylength experiment, the light intensities were adjusted so that each daylength regime received approximately the same amount of light energy per day. Differences demonstrated by the algae in the various incubators could then be attributed to differences in the daylength regime and not to total light effects.

The light intensity was held constant at approximately 200–250 ft c incubator experiments 4A and 4B. Maximum and minimum daylength and temperature values were used for each parameter, and incubators were set up with the four possible combinations of the two parameters. A low temperature combined with a short daylength corresponded approximately to winter values; a high temperature and a long daylength corresponded approximately to summer values in nature for the two parameters. The remaining two permutations, a low temperature combined with a long daylength and a high temperature with a short daylength, were completely artificial as far as natural occurrence, but the combination of the two parameters in the four ways possible permitted an assessment

of the role of the maximum and minimum seasonal values of temperature and daylength in culture.

The minimum and maximum temperatures were 13 C and 25 C, respectively, in both incubator experiments 4A and 4B. The former corresponds closely to the minimum mean monthly surface water temperature for Port Aransas in January. The maximum temperature employed, 25 C, does not correspond to the maximum mean monthly surface water temperature for Port Aransas, which is 30 C in July. A lower temperature was chosen for the maximum culture temperature for two reasons. First, the algal vegetation in mid-summer appears to decline slightly relative to that of late May and June; 25 C was selected, since it corresponds to the mean monthly surface water temperature for May when the summer vegetation was growing rapidly. Secondly, the salinity of the medium in the Petri dishes does not increase as much with time at 25 C as at 30 C.

The minimum and maximum daylength regimes varied in the two experiments. The maximum difference in mean monthly daylength between summer and winter in the Port Aransas area is only 3.5 hr. The daylength difference was increased to 8 hr, with minimum and maximum daylengths of 8 and 16 hr, respectively, in incubator experiment 4A. Short and long daylengths of 10 and 14 hr, respectively, which correspond closely to the minimum and maximum monthly daylength regimes in nature at Port Aransas, were subsequently employed in incubator experiment 4B. In addition, a 12-hr daylength regime at 25C was incorporated into the latter experiment.

The experimentation times for a particular alga may vary in different incubators, since the times given represent the time for a completed number of generations in each case.

Eight experiments were conducted with the cross-gradient culture apparatus, as follows:

Ectocarpus siliculosus—14-hr daylength
Ceramium strictum—14-hr daylength
Centroceras clavulatum—14-hr daylength
Polysiphonia boldii
- Tetrasporophytic phase—8-hr daylength
- Tetrasporophytic phase—14-hr daylength
- Carposporophytic phase—8-hr daylength
- Carposporophytic phase—14-hr daylength (repeated)

A temperature gradient of nearly 20 C from approximately 10 to 30 C and a light intensity gradient from approximately 50 to 500 ft c were employed. The temperature and light intensity values varied slightly in the various experiments; the values for the *Polysiphonia boldii* experiments are presented in Figure 61, and the values for the remaining experiments are given with the results.

Inocula and Source of Cultured Algae

Equal amounts of each alga were inoculated into the Petri dishes for a given experiment; the inoculum of a given alga may have varied slightly between different experiments.

The algae were maintained as stock-cultures, and from these the inocula for

the various experiments were derived. *Ectocarpus siliculosus, Petalonia fascia* and *Porphyra leucosticta* were cultured in a short daylength of 8 or 10 hr at 13 C with 50–200 ft c daylight fluorescent illumination. The remaining algae studied, including the *Conchocelis* phases of *Bangia fuscopurpurea* and *Porphyra leucosticta*, were maintained under longer daylengths of 12–16 hr at 20–25 C with 50–200 ft c daylight or cool-white fluorescent illumination. The algae were transferred directly from the stock-culture to the experimental regime in each instance.

The following twelve brown and red algae were cultured during the study. The source of the culture of each species is given below.

Phaeophycophyta

Ectocarpus siliculosus was attached to an oyster in shallow water at Rockport, Aransas Bay, in January 1968. Inoculum, four germlings per dish.

Giffordia mitchelliae was collected on the southwest jetty, Port Aransas, in April 1967. Inoculum, four germlings per dish.

Petalonia fascia was collected on the southwest jetty at Port Aransas, in January 1968. Inoculum, several plurilocular organ swarmers per dish.

Rhodophycophyta (Bangiophycidae)

Goniotrichum alsidii was found as an epiphyte on a larger alga in Redfish Bay, in January 1967. Inoculum, several monospores per dish.

Erythrocladia subintegra was collected as an epiphyte on *Padina vickersiae* on the southwest jetty, at Port Aransas, in January 1967. Inoculum, several monospores per dish.

Three isolates of *Bangia fuscopurpurea* were cultured, which were collected on the southwest jetty, Port Aransas, as follows: isolate 1 in April 1967; isolate 2 in May 1968; and isolate 3 in December 1968. Inoculum, 2–4 uniseriate germlings, or several α spores per dish.

The *Conchocelis* phase was produced exclusively by the filamentous phase of isolate 3 when the alga from nature was placed at 25 C with 205 ± 55 ft c (Fig. 29) daylight fluorescent illumination under a 14-hr daylength. When the filamentous phase was placed in the same temperature and light conditions, but under a 10-hr daylength, the resultant progeny was the filamentous phase except for a single *Conchocelis* plant. The inoculum for the *Conchocelis* phase comprised two small vegetative portions of the thallus per dish. The terminology used for the spores is the same as for *Porphyra*, discussed below.

Two isolates of *Porphyra leucosticta* were cultured, both of which were collected on the southwest jetty, Port Aransas, as follows: isolate 1 in January 1967; and isolate 2 in January 1968. The inoculum for the blade phase consisted of 2–4. small vegetative blades or several α spores per dish.

The *Conchocelis* phase used in incubator experiments 1–4A was produced by isolate 2 at 20–22 C, with 5–150 ft c cool white fluorescent illumination under a 12-hr daylength regime. The *Conchocelis* phase used in incubator experiment 4B was produced by isolates 1 and 2 of the blade phase earlier in the same experi-

ment. The inoculum for the *Conchocelis* phase consisted of two small vegetative portions of the thallus per dish.

Controversy currently exists concerning the presence of sexuality in the genus. At the present time it seems best to follow Conway (1964) who called the large pigmented spores produced by the blade phase α spores (often referred to as monospores or carpospores, depending on whether they reproduce the blade phase or give rise to the *Conchocelis* phase, respectively) and the smaller colorless spores β spores (often referred to as spermatia). The terminology used by various authors to describe the spores produced by the *Conchocelis* phase is also confused. The terminology of Dring (1967) is followed; he called the spores that produce the blade phase, conchospores, formed in conchosporangial branches (often referred to as fertile cell rows) and the spores that repeat the *Conchocelis* phase, monospores.

Rhodophycophyta (Florideophycidae)

Acrochaetium crassipes appeared as a contaminant in the culture of *Centroceras clavulatum* in January 1967. Inoculum, several monospores per dish.

Acrochaetium flexuosum was collected as an epiphyte on *Spyridia aculeata* on the southwest jetty, Port Aransas, in March 1967. Inoculum, two vegetative germlings per dish.

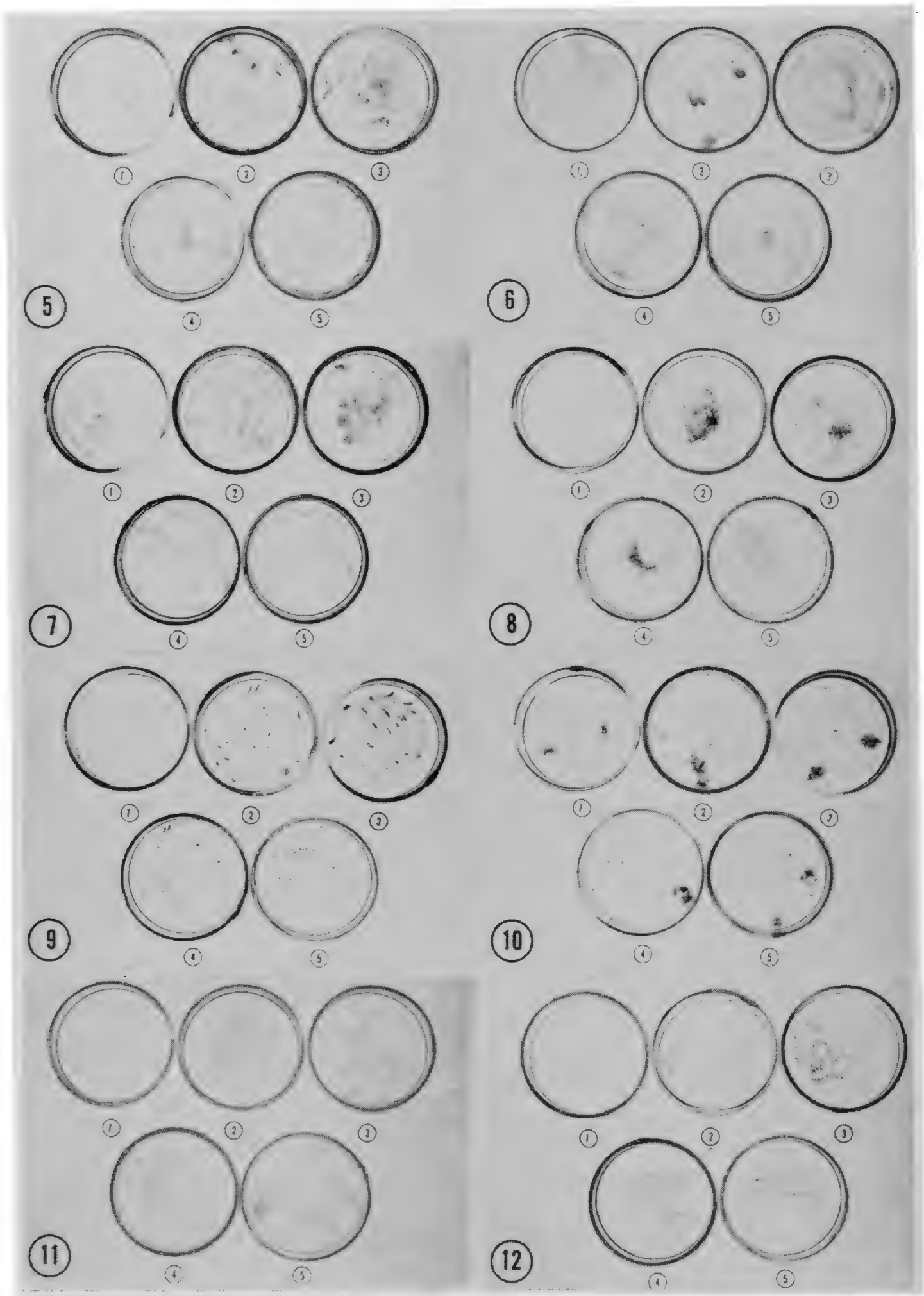
Ceramium strictum was collected on the southwest jetty, Port Aransas, in November 1966. Inoculum, four excised branch apices per dish. In the cross-gradient culture apparatus experiment six apices were initially used for the inoculum, but as growth proceeded, the healthiest four plants were retained.

Centroceras clavulatum was collected on the southwest jetty, Port Aransas, in November 1966, as a tetrasporophyte. The tetraspores were released and germinated to produce plants identical in morphology to the parent plant, but were still vegetative more than two years later. Inoculum, four excised branch apices of the tetrasporophyte phase. In the cross-gradient culture apparatus experiment six apices were initially used, but only the four healthiest plants were later retained.

Polysiphonia boldii was collected on the southwest jetty, Port Aransas, in July 1967. The life history of the isolate was reported in an earlier communication as *P. denudata* (Edwards 1968). The alga was originally identified as *P. denudata* by the author and this was confirmed by Dr. W. R. Taylor (*pers. comm.*), but further experience with the genus indicates that the species is closely related to *P. denudata* but is new to science. The inoculum comprised 4–8 germlings per dish. Germlings were used, rather than spores, since the latter were not always viable. Care was taken to insure the selection of healthy germlings since stunted plants frequently resulted from germlings of an unhealthy appearance. After some growth had taken place, the four healthiest plants in each dish were retained to continue the experiment.

RESULTS

The results are presented under three categories, namely: incubator experi-



FIGS. 5–12. The growth responses of eight marine algae to variations in light intensity, as investigated in incubator experiment 1. The numbers in the photographs refer to the light intensity regimes employed.

ments, cross-gradient culture apparatus experiments, and life-history observations in culture of *Polysiphonia boldii.*

Incubator Experiments

The five experiments are summarized below.

Experiment 1—Effect of Light Intensity

The parameters used in this experiment are presented in Table 1. Two dishes of each alga were placed in incubators 1–3, one dish in incubators 4–5, with the exception of *Polysiphonia boldii* for which three dishes of each spore type were placed in incubators 1–3, and two dishes in incubators 4–5. The experiment ran 29 days, again with the exception of *Polysiphonia boldii,* which was left in the incubator for 39 days. The medium was changed every 14 days. The algae responded to various light intensities as follows (Figs. 5–12).

Giffordia mitchelliae: growth increased with an increase in light intensity and was optimal at 140 ft c (Fig. 5). Plurilocular organs were observed only at intensities less than 50 ft c; they almost certainly developed under the higher light intensity regimes, but had discharged since they were the only type of reproductive organ ever observed in the isolate and germlings were observed in the dishes.

Erythrocladia subintegra: similar amounts of growth (Fig. 6) and monospore formation were present under all regimes tested.

Goniotrichum alsidii: similar amounts of growth took place under all regimes tested (Fig. 7). The plants became pluriseriate and formed monospores.

Bangia fuscopurpurea (Isolate 1): growth increased with an increase in light intensity and was optimal at 140 ft c (Fig. 8). Alpha spores were formed under all regimes tested; β spores did not form.

Porphyra leucosticta (Isolate 1):
Blades: the growth of the blades increased with an increase in light intensity and was optimal at 140 ft c. The blades remained small and had crinkly edges; α spores formed under all light intensities tested. Beta spores did not form at 50 ft c but were present under the higher light regimes.
Conchocelis: similar growth responses occurred under all regimes tested. One swollen cell on the filament was observed at 240 and 400 ft c.

Acrochaetium crassipes: the growth increased with an increase in light intensity

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FIG. 5. *Giffordia mitchelliae.*
FIG. 6. *Erythrocladia subintegra.*
FIG. 7. *Goniotrichum alsidii.*
FIG. 8. *Bangia fuscopurpurea.*
FIG. 9. *Acrochaetium crassipes.*
FIG. 10. *Acrochaetium flexuosum.*
FIG. 11. *Ceramium strictum.*
FIG. 12. *Centroceras clavulatum.*

TABLE 1

Parameters employed to investigate the effects of light intensity

Parameter	Incubator 1	2	3	4	5
Light intensity (ft c)	50±5	140±20	240±20	400±50	635±65
Daylength (hr)	14	14	14	14	14
Temperature (C)	25±0.5	25±0.5	25±0.5	25±0.5	25±0.5

and was optimal at 140 ft c (Fig. 9). Monospore formation took place under all regimes.

Acrochaetium flexuosum: the response was similar to that of *A. crassipes* (Fig. 10).

Ceramium strictum: the growth increased with an increase in light intensity and was optimal at 140 ft c (Fig. 11). The plants were vegetative.

Centroceras clavulatum: the growth increased with an increase in light intensity and was optimal at 400 ft c (Fig. 12). The plants were vegetative.

Polysiphonia boldii: growth responses of gametophytes and tetrasporophytes were similar. Growth proceeded slowly at 50 ft c, was optimal at 140, 240 and 400 ft c for the gametophytic phase and at 140 and 240 ft c for the tetrasporophytic phase. The growth was slightly below optimal at 635 ft c for the gametophytic phase, and at 400 and 635 ft c for the tetrasporophytic phase. Tetrasporangial formation and tetraspore release took place in all light intensities tested with a maximum at 140 and 240 ft c. Formation of antheridia and carpogonia took place in all light intensities tested, carposporophytic development at 240 and 400 ft c and carpospore release at 140 ft c.

The red algae showed a tendency to become brownish under higher light intensities.

The algae tested fell into three categories with regard to their response to variations in light intensity:

(1) those that responded similarly to all the light intensity regimes employed and were thus light-saturated at very low intensity values (*e.g., Goniotrichum alsidii* and the *Conchocelis* phase of *Porphyra*); (2) those that demonstrated an increase in growth with an increase in light intensity until and optimal intensity was reached, when the growth "levelled off" (*e.g., Giffordia mitchelliae* and *Centroceras clavulatum*); and (3) those whose growth increased with an increase in light intensity to an optimal level, after which the growth declined slightly under the higher light intensity regimes employed (*e.g., Polysiphonia boldii*).

Experiment 2—Effect of Daylength

The parameters used are presented in Table 2. The light intensities were adjusted so that the plants at various photoperiods received approximately equal amounts of light energy per day. Since light intensity has little apparent effect on growth, as shown in the preceding experiment, the light intensities were ad-

TABLE 2

Parameters employed to investigate the effects of daylength

Parameter	Incubator 1	2	3	4	5
Daylength (hr)	8	12	16	20	24
Light intensity (ft c)	285±15	190±10	140±10	120±10	100±10
Temperature (C)	23.5±0.5	23.5±0.5	23.5±0.5	23.5±0.5	23.5±0.5

justed to eliminate any total light effects. Each incubator contained three dishes of each algae. Most of the algae were incubated for 27 days; however, *Giffordia mitchelliae* was removed after 18 days, while *Polysiphonia boldii* ran 42 days and *Porphyra* (blade) and *Porphyra* (*Conchocelis*) ran 53 days. The medium was changed every 14 days.

The responses of the algae to the different daylengths were as follows (Figs. 13–20).

Giffordia mitchelliae: growth was similar and plurilocular organs formed under all daylength regimes tested (Fig. 13).

Erythrocladia subintegra: growth was similar and monospores formed under all daylength regimes tested (Fig. 14).

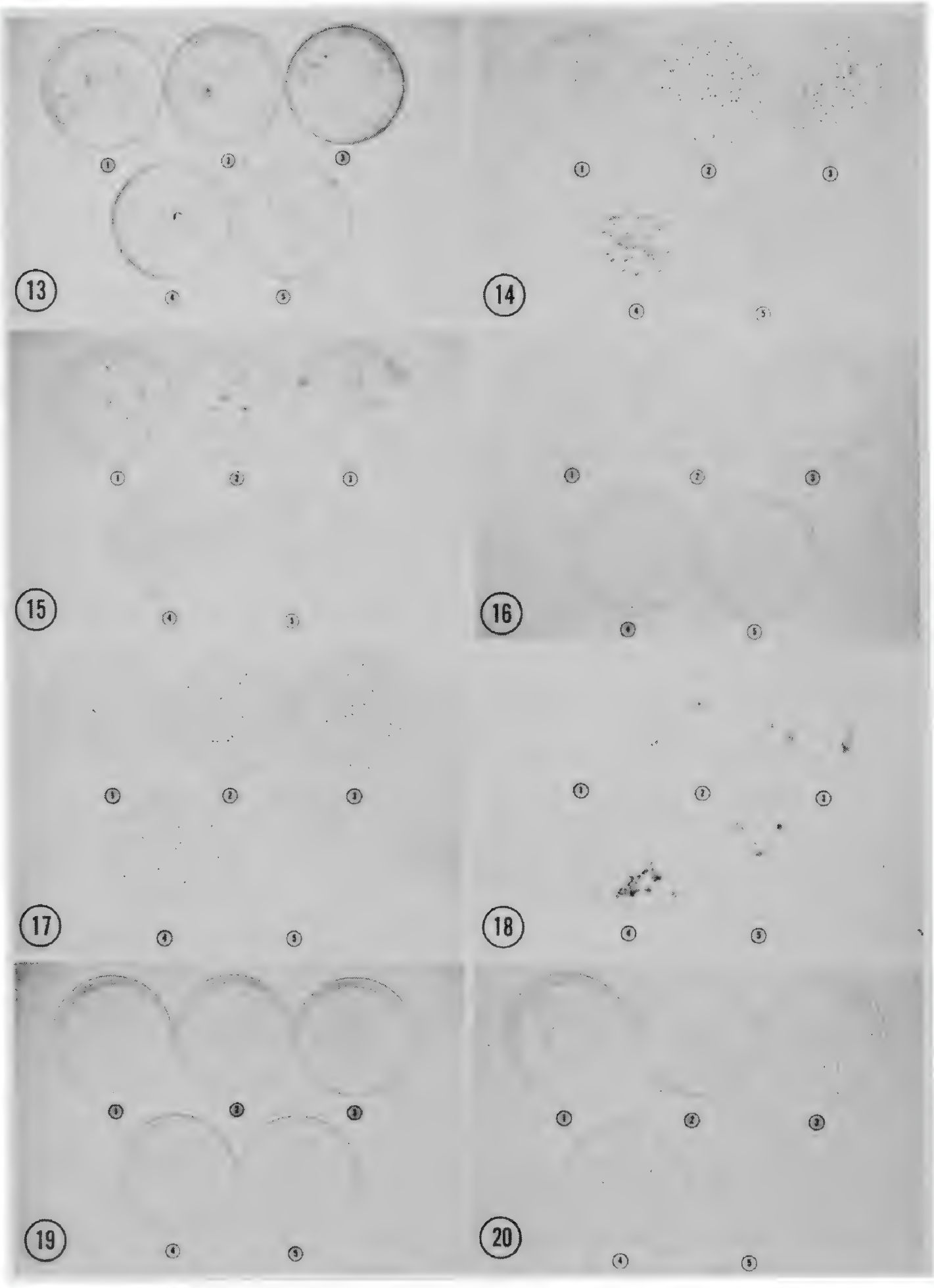
Goniotrichum alsidii: good growth resulted under daylengths of 12-, 16-, 20- and 24-hr, but growth was slower under an 8-hr daylength (Fig. 15). The plants became pluriseriate and formed monospores under all photoperiods.

Bangia fuscopurpurea (Isolate 1): growth was fair under daylengths of 8-, 12-, 16- and 20-hr, but poor under continuous light (Fig. 16); α spores formed under all daylength regimes, but β spores were not observed.

Porphyra leucosticta (Isolate 1):
Blades: the progeny which resulted under the various photoperiods are as follows: 8-hr—blades; 12-hr—blades with a few *Conchocelis* plants; 16-hr—approximately equal numbers of blades and *Conchocelis* plants; 20-hr—mainly *Conchocelis* plants with a few blades; 24-hr—mainly blades with a few *Conchocelis* plants.

The types of spores formed by the original blades used for the inoculum could not be determined since the blades had disintegrated through sporulation before they were examined. However, many of the *Conchocelis* plants could be seen to be derived from large pigmented α spores.

Conchocelis: similar growth responses occurred under all the daylength regimes tested, but conchosporangial branches formed under the 8-hr photoperiod (Fig. 30); thickened filaments had started to develop under the 12-hr daylength regime (Fig. 31), but the *Conchocelis* from the longer daylength regimes remained completely vegetative (Fig. 32). An increase in the number of *Conchocelis* plants per dish took place under all daylength regimes, but this was probably due to fragmentation since the increase in number was small and no reproductive struc-



FIGS. 13–20. The growth responses of eight marine algae to variations in daylength, as investigated in incubator experiment 2. The numbers in the photographs refer to the daylength regimes employed.

tures at all were observed on the *Conchocelis* plants from the longer daylength regimes.

Acrochaetium crassipes: good growth occurred under regimes of 12-, 16- and 20-hr daylength. Growth was slightly less than optimal under continuous light, and still further below optimal under 8-hr daylength (Fig. 17). Monospore formation took place under all daylength regimes tested.

Acrochaetium flexuosum: good growth occurred under photoperiods of 12-, 16-, 20- and 24-hr, but growth was slow under a daylength of 8-hr (Fig. 18). Monospore formation occurred under all daylength regimes tested.

Ceramium strictum: good growth occurred under all daylength regimes. Growth was optimal under daylength of 16- and 20-hr; slightly below optimal under daylengths of 12- and 24-hr; and still further below optimal under a daylength of 8-hr (Fig. 19). The plants were vegetative.

Centroceras clavulatum: growth was optimal under daylengths of 8-, 12-, 16- and 20-hr and slightly below optimal under a daylength of 24-hr (Fig. 20). The plants were vegetative.

Polysiphonia boldii: growth responses of gametophytes and tetrasporophytes were similar. Good growth was demonstrated by both phases under all photoperiods tested, but the growth of the gametophytic phase under an 8-hr daylength was slightly less than under the other daylength regimes. Tetrasporangia did not form under short daylengths of 8-hr or in continuous light; they developed under 12-, 16- and 20-hr daylengths, with maximum formation occurring under the latter two regimes. Antheridial and carpogonial formation and carposporophytic development took place under all photoperiod regimes tested; carpospores were released under the 12-, 16- and 20-hr daylength regimes.

Varying growth responses to the different daylength regimes were apparent. *Giffordia mitchelliae* and *Erythrocladia subintegra* demonstrated good growth independently of the daylength regime employed. The growth of *Goniotrichum alsidii* and *Acrochaetium flexuosum* was slow under an 8-hr photoperiod, while that of *Bangia fuscopurpurea* was inhibited by continuous light.

Variations in daylength had pronounced effects on reproduction in several instances. *Giffordia mitchelliae* and *Erythrocladia subintegra* reproduced under all daylength regimes, but *Porphyra* (blade), *Porphyra* (*Conchocelis*) and *Polysiphonia boldii* responded differently to varying daylength regimes. The formation of *Conchocelis* by *Porphyra* blades increased with an increase in daylength

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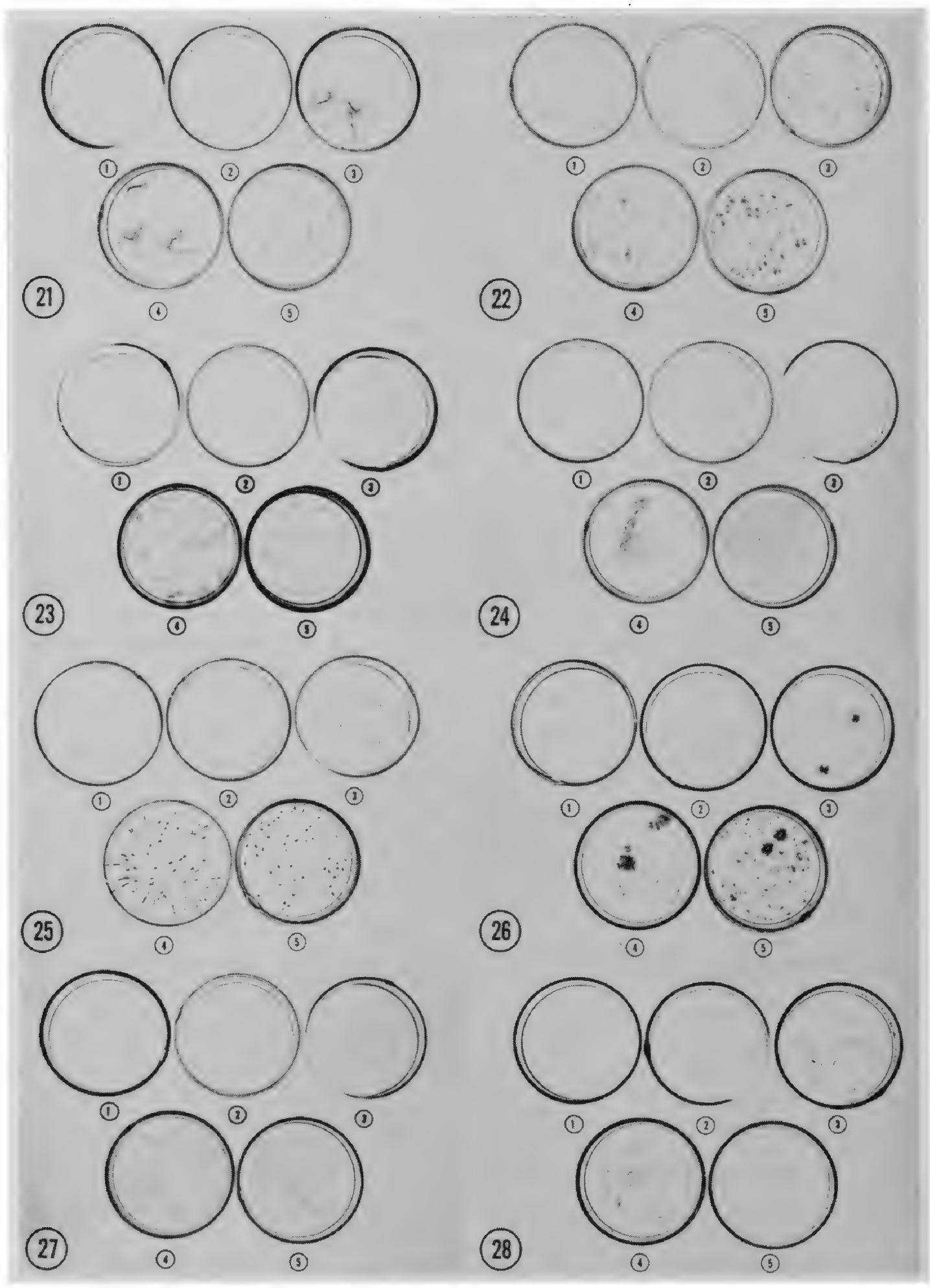
FIG. 13. *Giffordia mitchelliae.*
FIG. 14. *Erythrocladia subintegra.*
FIG. 15. *Goniotrichum alsidii.*
FIG. 16. *Bangia fuscopurpurea.*
FIG. 17. *Acrochaetium crassipes.*
FIG. 18. *Acrochaetium flexuosum.*
FIG. 19. *Ceramium strictum.*
FIG. 20. *Centroceras clavulatum.*



FIGS. 21–28. The growth responses of eight marine algae to variations in temperature, as investigated in incubator experiment 3. The numbers in the photographs refer to the temperature regimes employed.

TABLE 3

Parameters used to investigate the effects of temperature

Parameter	Incubator 1	2	3	4	5
Temperature (C)	9.5±0.5	14.5±0.5	19±1	24.5±0.5	20.5±0.5
Light intensity (ft c)	175±35	175±35	175±35	175±35	175±35
Daylength (hr)	14	14	14	14	14

from a 12-hr, through 16-hr, to a 20-hr daylength. Only a small amount of *Conchocelis*, relative to the number of young blades, developed in continuous light, but under a short daylength (8-hr) only young blades developed. The *Conchocelis* phase developed conchosporangial branches under a short daylength of 8-hr but blades were not produced. Daylength had a profound effect on reproduction in *Polysiphonia boldii*. The formation of antheridia and carpogonia, fertilization and carposporophytic development took place under all daylengths tested, but tetrasporangia did not form under short daylengths of 8-hr or in continuous light.

Experiment 3—Effect of Temperature

The parameters used are presented in Table 3. Two dishes of each alga were placed in the incubators. The experiment ran a total of 47 days. *Giffordia mitchelliae* was incubated for 23 days; *Erythrocladia subintegra*, *Goniotrichum alsidii*, *Bangia fuscopurpurea*, *Acrochaetium crassipes* and *Ceramium strictum* for 25 days; *Porphyra* (blade), *Porphyra* (*Conchocelis*), *Acrochaetium flexuosum* and *Centroceras clavulatum* for 32 days; and *Polysiphonia boldii* for 47 days. The medium was changed every 7–14 days.

The algae responded to the various temperatures as follows (Figs. 21–28).

Giffordia mitchelliae: no growth and a loss of pigmentation of the inoculum occurred at 9.5 C. Growth increased with an increase in temperature; optimal growth was at 24.5 C. Growth at 29.5 C was slow (Fig. 21). Plurilocular organs developed under all temperatures at which growth took place.

Erythrocladia subintegra: at 9.5 C the monospores used for the inoculum germinated, but most of the resulting plants later showed a loss of pigmentation. Growth and monospore formation proceeded slowly at 14.5 C, but at 19, 24.5 and 29.5 C growth, which was accompanied by monospore formation, was optimal (Fig. 22).

Goniotrichum alsidii: growth did not occur at 9.5 and 14.5 C. Growth and mono-

FIG. 21. *Giffordia mitchelliae.*
FIG. 22. *Erythrocladia subintegra.*
FIG. 23. *Goniotrichum alsidii.*
FIG. 24. *Bangia fuscopurpurea.*
FIG. 25. *Acrochaetium crassipes.*
FIG. 26. *Acrochaetium flexuosum.*
FIG. 27. *Ceramium strictum.*
FIG. 28. *Centroceras clavulatum.*

spore formation proceeded slowly at 19 C and increased to a maximum at 24.5 and 29.5 C (Fig. 23).

Bangia fuscopurpurea (Isolate 1): no growth and a loss of pigmentation of the inoculum occurred at 9.5 C. Growth and the formation of α spores increased with an increase in temperature, to reach a maximum at 24.5 and 29.5 C; β spores did not develop (Fig. 24).

Porphyra leucosticta (Isolate 1):
Blades: growth of the small blades that comprised the inoculum occurred at all temperatures tested, except 29.5 C; there the blades lost their pigmentation. At 9.5 C, the original blades grew slowly and assumed a healthy pinkish-red coloration, but remained vegetative. At 14.5, 19 and 24.5 C, the original blades became brownish-red and the margins of the blades became crinkled. They did not grow as large as did the blades at 9.5 C, but all sporulated. The resulting progeny consisted largely of new blades, but a few *Conchocelis* individuals formed at each of the three temperatures. The types of spores formed by the original blades were not ascertained, since the blades had disintegrated through sporulation before they were examined. New blades sporulated under each of the three temperature regimes, but the degree of sporulation increased from 14.5 through 19 to 24.5 C; α and β spores were formed by the blades under each of the three temperature regimes.

Conchocelis: good growth occurred at 14.5, 19 and 24.5 C. At 9.5 and 29.5 C, some growth did take place, but it was much less than at the intermediate temperatures. At 14.5 and 19 C, one swollen cell and one thickened portion of a filament resembling a conchosporangial branch, occurred at each temperature tested. At 14.5, 19 and 24.5 C, there was a small increase in the number of *Conchocelis* individuals, but this was probably due to fragmentation of the original plants.

Acrochaetium crassipes: growth did not occur at 9.5 C. Growth took place slowly at 14.5 C and then increased with temperature until a maximum was reached at 24.5 and 29.5 C (Fig. 25). Monospore formation took place between 14.5 and 29.5 C.

Acrochaetium flexuosum: no growth took place at 9.5 C. Growth proceeded slowly at 14.5 C and then increased with temperature to a maximum at 29.5 C (Fig. 26). Monospores formed between 14.5 and 29.5 C.

Ceramium strictum: the inoculum did not grow at 9.5 C and underwent loss of pigmentation. Slight growth took place at 14.5 C, but the plants were stunted and became brownish-red. Good growth with normal morphology and pigmentation occurred at 19, 24.5 and 29.5 C, with maximum growth taking place at 24.5 and 29.5 C (Fig. 27). The plants were vegetative.

Centroceras clavulatum: no growth took place at 9.5 and 14.5 C. The inoculum lost its pigment at 9.5 C and became brownish-red at 14.5 C. Good growth with normal morphology and pigmentation occurred at 19 and 24.5 C; growth was much faster at 24.5 than at 19.5 C. At 29.5 C, a small amount of growth took place and the plants were stunted (Fig. 28). The plants were vegetative.

Polysiphonia boldii: growth responses of gametophytes and tetrasporophytes were similar. Growth did not occur at 9.5 C. Growth proceeded slowly at 14.5 C and then increased with an increase in temperature until optimal growth was attained at 24.5 and 29.5 C. Tetrasporangial formation and release took place at 19 and 24.5 C, but the plants remained vegetative when tested at the lower and higher temperatures at which growth occurred. Antheridial and carpogonial formation took place between 14.5 and 29.5 C, in addition to carposporophytic development and carpospore release between 19 and 29.5 C.

The majority of the algae investigated did not grow at the lowest temperature tested, 9.5 C. However, the *Conchocelis* and blade phases of *Porphyra* both grew slowly at this temperature. Most algae commenced growth at 14.5 C and showed an increase in growth with temperature before reaching an optimum at the higher temperatures utilized in the experiment; *e.g., Erythrocladia subintegra* and *Ceramium strictum. Giffordia mitchelliae* and *Centroceras clavulatum* demonstrated a decrease in growth at 29.5 C, indicating that this temperature was higher than their optimum growth temperature. *Giffordia mitchelliae* and *Acrochaetium flexuosum* reproduced at all temperatures at which growth took place. In *Polysiphonia boldii* sexual organs developed when growth occurred, but tetrasporangia did not develop at 14.5 and 29.5 C, the lowest and highest temperatures, respectively, at which growth took place.

Experiment 4A—Effect of Simultaneous Variations of Daylength and Temperature

The parameters used in experiment 4A are presented in Table 4. These same parameters prevailed for the study of all the algae in the experiment and are thus not repeated further.

Three dishes of each alga were placed in the different regimes. The medium was replenished after 6–13 days, but usually every 7 days. The experiment ran a total of 159 days.

Porphyra leucosticta (Isolate 2): the results with the isolate are presented in Table 5.

The growth rate of the blades was much faster and sporulation took place much earlier at 25 C than at 13 C. Sporulation occurred when the blades were quite small at 25 C, but at 13 C the blades attained a much larger size before reproducing. The blades grew larger and took longer to reproduce under the 8-hr than the 16-hr daylength regime at 13 C, but at 25 C daylength differences were not ap-

TABLE 4

Parameters employed in experiment 4A to investigate the effects of simultaneous variations of daylength and temperature

Parameter	Incubator 1	2	3	4
Daylength (hr)	8	16	8	16
Temperature (C)	13±0.5	13±0.5	25±0.5	25±0.5
Light intensity (ft c)	220±40	220±40	220±40	220±40

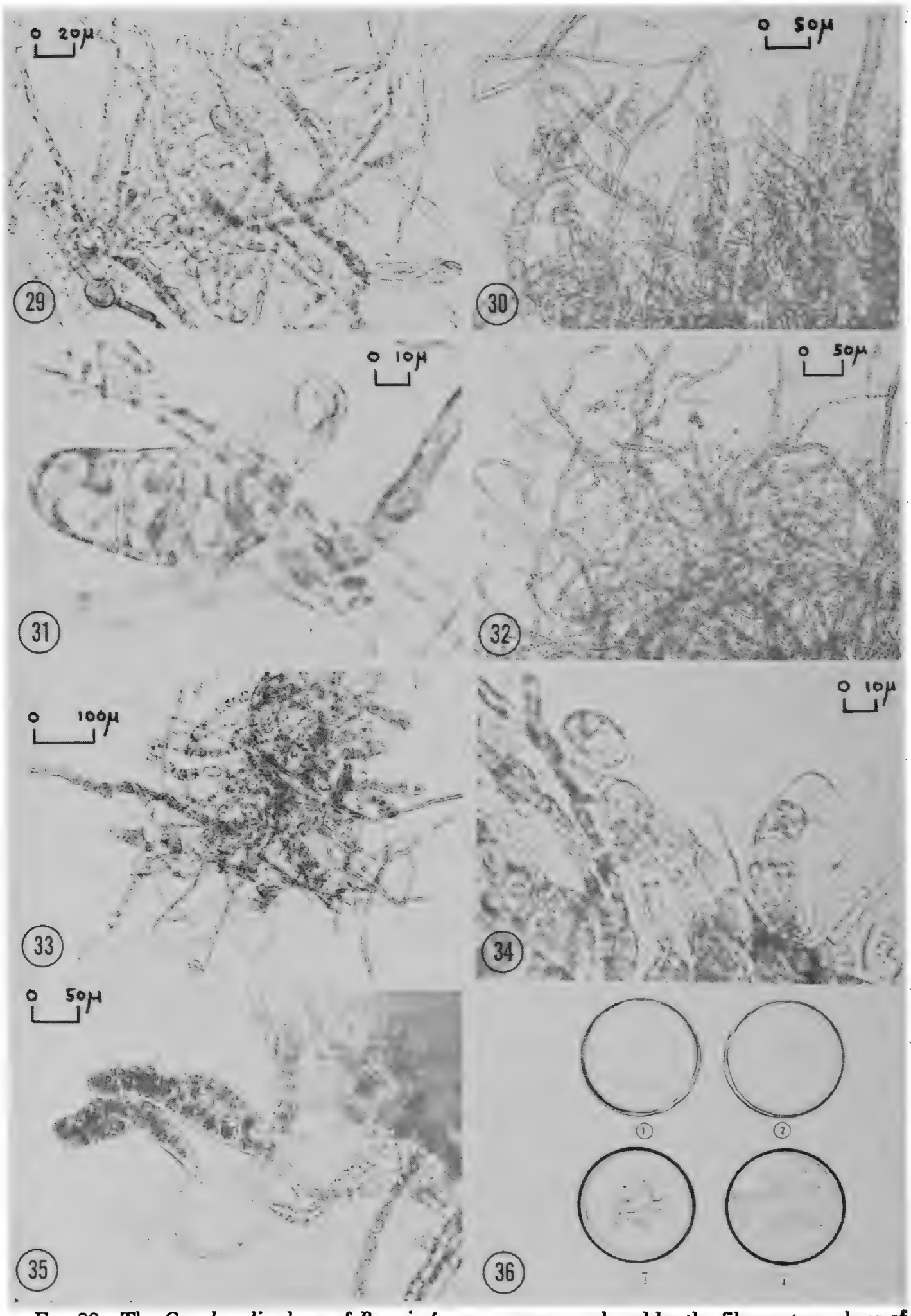


FIG. 29. The *Conchocelis* phase of *Bangia fuscopurpurea* produced by the filamentous phase of isolate 3 when the alga from nature was cultured at 25 C with 205 ft c illumination under a 14-hr daylength. The microscopic *Conchocelis* phase developed from the large pigmented α spores of the filamentous phase, which may still be seen.

TABLE 5

Summary of the results of experiment 4A with *P. leucosticta* (Isolate 2).

Parameter	Incubator 1	2	3	4
Experimentation time (days)	138	145	145	151
Number of generations	2	3	7	8
Average generation time (days)	69	48.3	20.7	18.9
Spore types formed	α,β	α,β	α	α,β
Resultant progeny	blades	blades	blades	blades

parent. Alpha spores formed abundantly under all four regimes but β spores were produced in abundance only at 13 C; a few β spores developed at 25 C under the 16-hr daylength, but were absent under the 8-hr daylength regime.

The blades assumed a brownish-red coloration and had crinkly margins at 25 C, but at 13 C were reddish-purple and twisted, without crinkly margins.

The *Conchocelis* phase did not develop under any of the four regimes.

Porphyra leucosticta (*Conchocelis* phase, Isolate 1): the *Conchocelis* phase remained healthy under all regimes tested, but grew more rapidly at 25 C than at 13 C. No growth differences could be detected between the two daylength regimes at either temperature. A small number of *Conchocelis* plants appeared in each dish under the various regimes, but they probably arose by fragmentation of the original inoculum.

The experiment ran 151 days. *Conchocelis* remained vegetative under the 16-hr daylength regime at 25 C and at 13 C. However, the *Conchocelis* phase formed conchosporangial branches (Figs. 30–33) and blades under the 8-hr daylength regime at 25 C and 13 C. At 25 C, blades appeared in the three dishes of *Conchocelis* after 51, 58 and 84 days, whereas at 13 C blades appeared in one dish only, after 71 days. It appears that a short daylength regime of 8-hr is conducive to the formation of blades by *Conchocelis*, with the change taking place faster at 25 C than at 13 C.

FIG. 30. The conchosporangial branches produced by the *Conchocelis* phase of *Porphyra leucosticta*, isolate 1, in incubator experiment 2 under an 8-hr daylength after 53 days.

FIG. 31. Early development of a conchosporangial branch by the *Conchocelis* phase of *Porphyra leucosticta*, isolate 1, in incubator experiment 2 under a 12-hr daylength after 53 days.

FIG. 32. The vegetative *Conchocelis* phase of *Porphyra leucosticta*, isolate 1, in incubator experiment 2 under a 14-hr daylength after 29 days.

FIG. 33. The conchosporangial branches formed by the *Conchocelis* phase of *Porphyra leucosticta*, isolate 1, in incubator experiment 4A under an 8-hr daylength.

FIG. 34. The conchosporangial branches of the *Conchocelis* phase of *Porphyra leucosticta*, isolate 1, in incubator experiment 4A under an 8-hr daylength after 51 days. The protoplasts of some of the cells appear to have rounded up. The occurrence of empty cells indicates that conchospores may have developed.

FIG. 35. Young blades apparently attached to *Conchocelis* filaments of *Porphyra leucosticta*, isolate 1, in incubator experiment 4A under an 8-hr daylength after 51 days.

FIG. 36. The growth response of *Giffordia mitchelliae* to simultaneous variations of daylength and temperature in incubator experiment 4A. Numbers 1–4 designate the incubator regimes.

TABLE 6

Summary of the results of experiment 4A with *B. fuscopurpurea* (Isolate 2)

Parameter	Incubator 1	2	3	4
Experimentation time (days)	94	108	101	101
Number of generations	1	2	5	7
Average generation time (days)	94	54	20.2	14.4
Spore types formed	α	α	α	α
Resultant progeny	filaments	filaments	filaments	filaments

The precise manner in which the blade phase arose from *Conchocelis* could not be determined since the blades were formed only in limited numbers. Conchospores were not observed, but they probably formed since young blades developed some distance from the *Conchocelis* which had conchosporangial branches with several empty cells (Fig. 34). The blades occasionally appeared to be attached to the *Conchocelis* filament (Fig. 35) which suggests that they may have grown directly from the *Conchocelis* and not from liberated spores, as Krishnamurthy (1969) observed in *Porphyra cuneiformis*.

Bangia fuscopurpurea (Isolate 2): the results with the isolate are presented in Table 6. In general, the alga grew much faster and attained a larger size at 25 C than at 13 C, irrespective of daylength. The filaments grew long and were of small diameter at the higher temperature as compared with those at the lower temperature, which were usually much shorter and thickened. The alga at 25 C was healthy in appearance and comparable in morphology to plants found in nature, but at 13 C the plants were stunted and atypical in form. Alpha spores were the only spore type produced and they always repeated the filamentous phase. The generation time was much shorter at 25 C than at 13 C. A daylength effect was apparent in that the generation time was shorter under the 16-hr than under the 8-hr daylength regime and the difference was accentuated at 13 C relative to 25 C. The growth differences were probably total light effects due to the alga receiving a greater amount of light energy per day under the 16-hr than under the 8-hr daylength regime.

Bangia fuscopurpurea (Isolate 1): results are presented in Table 7. The results were similar to those with Isolate 2, but the growth responses were at variance.

TABLE 7

Summary of the results of experiment 4A with *B. fuscopurpurea* (Isolate 1)

Parameter	Incubator 1	2	3	4
Experimentation time (days)	52	38	52	52
Number of generations	—	1	4	4
Average generation time (days)	—	38	13	13
Spore types formed	—	α	α	α
Resultant progeny	—	filaments	filaments	filaments

The plants at 25 C grew more slowly with a 16-hr light period than with an 8-hr one and the filaments also tended to thicken under the longer daylength. At 13 C the plants grew quite well under the 16-hr daylength but tended to thicken, whereas under the 8-hr daylength the filaments grew slowly but did not thicken.

Giffordia mitchelliae: good growth, accompanied by plurilocular organ formation, took place at 25 C under both 8- and 16-hr daylength regimes. The plants grew a little more slowly under the 16-hr than under the 8-hr daylength, but sporulated more. They grew less at 13 C than at 25 C. A little more growth occurred under the 16-hr than under the 8-hr daylength regime at 13 C, but all the plants remained vegetative (Fig. 36).

Ectocarpus siliculosus: good growth with plurilocular organ formation occurred at 25 C under both 8- and 16-hr daylengths, but sporulation was greater under the latter daylength. Equal amounts of growth took place under 8- and 16-hr daylengths at 13 C, but growth was much less than at 25 C (Fig. 37). Plurilocular organs were not observed at 13 C but were almost certainly formed under both daylength regimes, since germlings were present in the dishes.

Petalonia fascia: early development of swarmers from the plurilocular organs of the blade was similar under the four daylength and temperature regimes tested.

Germlings were initially unbranched filaments, which later branched; the branches adhered laterally in varying degrees to produce filamentous aggregations (a limited amount of coalescence of filaments), which proponderated, or discs (extensive coalescence of filaments). This basal system subsequently developed upright parenchymatous blades (Figs. 38, 39).

The growth rate at 25 C was much faster than at 13 C. No difference in growth rate due to daylength variations was apparent.

Blades resembling those found in nature during winter and early spring, but of smaller size, were formed only at 13 C under an 8-hr daylength. Each basal system gave rise to blades and subsequently remained relatively small in relation to the blade. The latter later developed plurilocular organs after 65 and 94 days in the two experiments, respectively.

Similarly, progeny resulted in the remaining three daylength and temperature regimes. The basal systems (filamentous aggregations and discs) which were linked by a series of intermediate stages, grew much larger than those at 13 C under the 8-hr daylength and did not always give rise to upright blades but remained vegetative (Fig. 40). The blades that did develop remained small relative to those at 13 C under the 8-hr daylength regime and later became darkened and thickened. The blades developed plurilocular organs under all regimes tested, but much sooner than at 13 C under an 8-hr daylength regime.

Three portions of a filamentous aggregation, which had not produced blades after 76 days at 25 C under a 16-hr daylength, were placed in each of the other three daylength and temperature regimes. At 13 C and under the 8-hr daylength all three portions produced an abundance of blades in 16 days (Fig. 41). At 13 C and under the 16-hr daylength, a few blades had been formed by one portion of the filamentous aggregation in 21 days, but the other two remained unchanged.

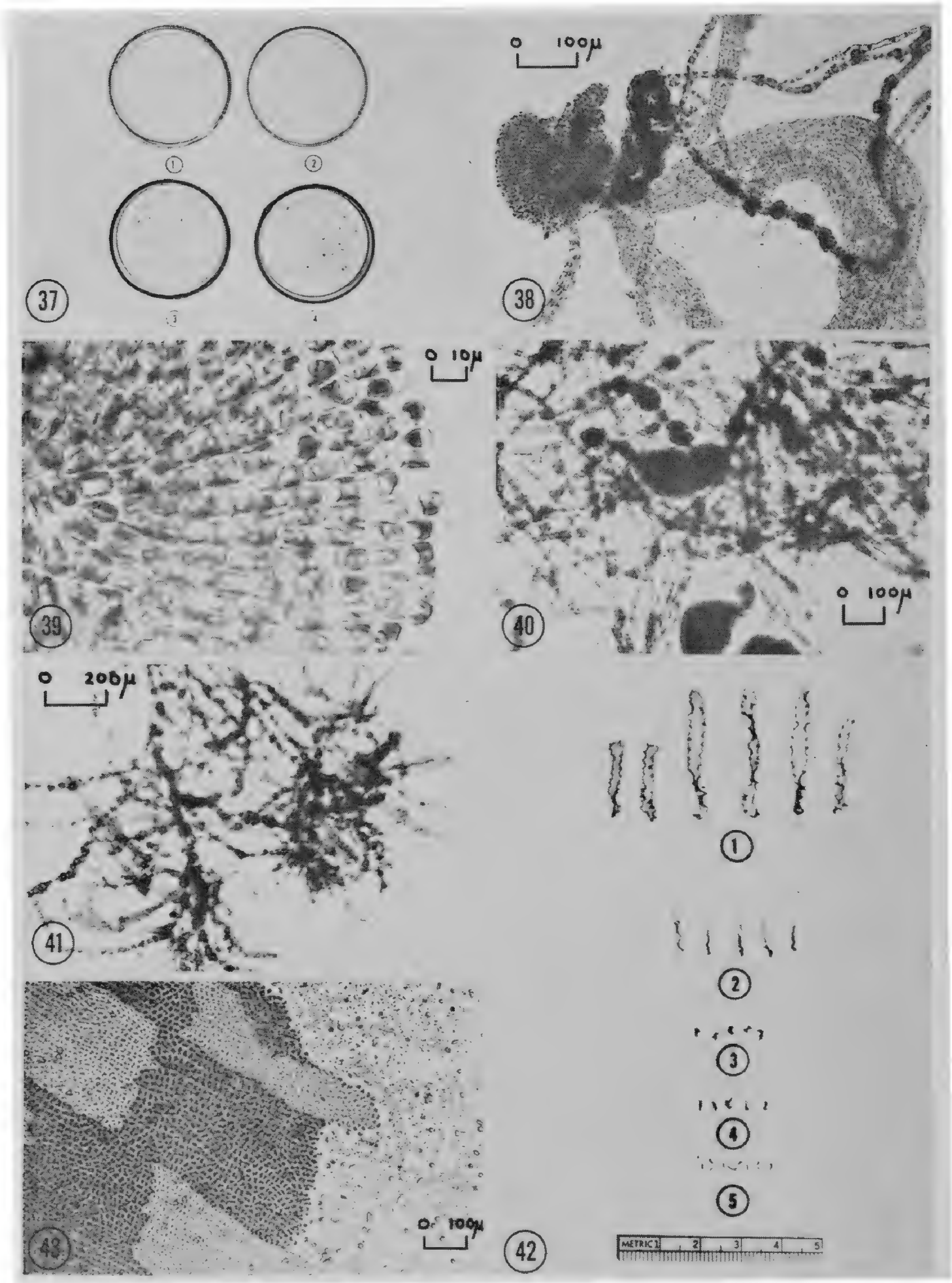


FIG. 37. The growth responses of *Ectocarpus siliculosus* to simultaneous variations of day-length and temperature in incubator experiment 4A.

FIG. 38. The basal system of *Petalonia fascia* giving rise to an upright blade at 13 C under an 8-hr daylength, after 52 days, in incubator experiment 4A. The basal system of the plant was predominantly discoidal, but was composed in part of filamentous aggregations.

TABLE 8

Parameters employed in experiment 4B to investigate the effects of simultaneous variations of daylength and temperature

Parameter	Incubator 1	2	3	4	5
Daylength (hr)	10	14	10	12	14
Temperature (C)	13	13	25	25	25[1]
Light intensity (ft c)	205±55	205±55	205±55	205±55	205±55

[1] The temperature rose to 35 C on one occasion, for nearly 30 min, but no apparent damage to the algae occurred.

At 25 C under an 8-hr daylength two filamentous aggregations sprouted blades and one remained unchanged in 14 days. Thus, only a combination of cool temperatures and short days caused an abundance of blades to be formed by all three portions of the filamentous aggregation.

Experiment 4B—Effect of Simultaneous Variations of Daylength and Temperature

The parameters used in the experiment are presented in Table 8. These same parameters prevailed for all the algae in the experiment and are not repeated further.

Two dishes of each alga were placed in the different regimes. The medium was replenished every 5–12 days, but usually after 7 days. The experiment ran a total of 148 days.

Porphyra leucosticta (Isolate 2): the results for the isolate are presented in Table 9. The results were very similar to those of the preceding experiment. However, the *Conchocelis* phase developed on two occasions in this experiment. One plant was produced in the second generation at 25 C under a 12-hr daylength and three plants were produced in the second generation at 25 C under a 14-hr daylength.

Porphyra leucosticta (Isolate 1): the results with the isolate are presented in Table 10. The results were similar to isolate 2.

FIG. 39. A close-up of part of the discoidal portion of the basal system of the plant in the preceding figure.

FIG. 40. A filamentous aggregation type of basal system which did not develop blades. It developed at 25 C under a 16-hr daylength, after 66 days in incubator experiment 4A.

FIG. 41. A filamentous aggregation type of basal system which developed at 25 C under a 16-hr daylength, sprouted blades in 16 days after being transferred to 13 C under an 8-hr daylength regime. Incubator experiment 4A.

FIG. 42. The growth responses of *Porphyra leucosticta*, isolate 1, to simultaneous variations of daylength and temperature in incubator experiment 4B. Numbers 1–5 designate the incubator regimes.

FIG. 43. The mosaic pattern of spore formation of *Porphyra leucosticta, isolate* 1, at 13 C under an 8-hr daylength in incubator experiment 4B. Regions of the blade form large patches of large pigmented α spores or smaller colorless β spores.

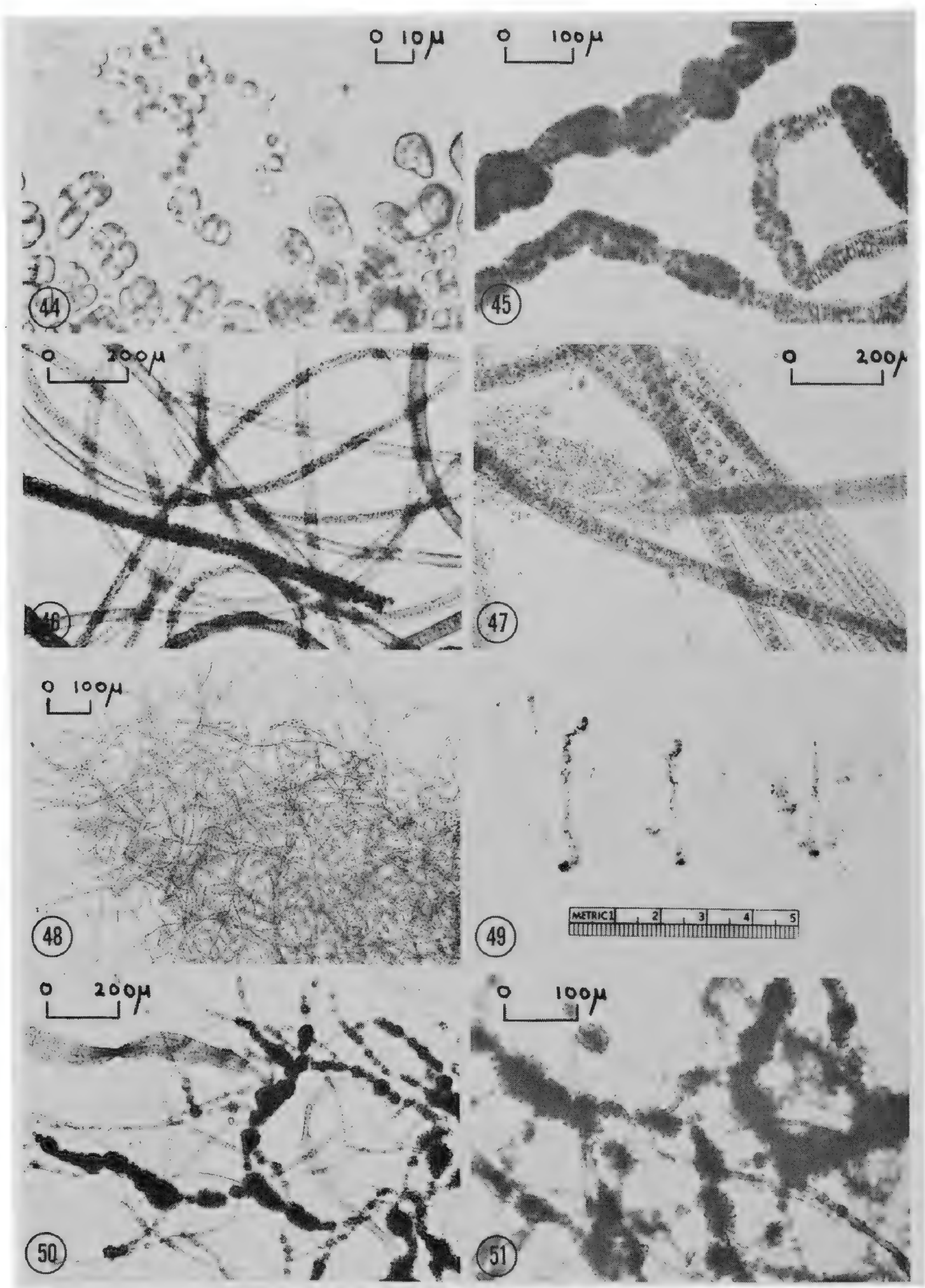


FIG. 44. The α and β spores of the blade in Fig. 43, highly magnified.

FIG. 45. The stunted filaments of relatively large diameter of *Bangia fuscopurpurea*, isolate 2, formed at 13 C under a 14-hr daylength, in incubator experiment 4B.

FIG. 46. The elongated filaments of relatively small diameter of *Bangia fuscopurpurea*, isolate

TABLE 9

Summary of the results of experiment 4B with *P. leucosticta* (Isolate 2)

	Incubator				
	1	2	3	4	5
Experimentation time (days)	65	100	100	86	100
Number of generations	1	2	5	3	5
Average generation time (days)	65[1]	50[2]	20	28.7	20
Spore types formed	α,β	α,β	α	α,β	α,β
Resultant progeny	blades	blades	blades	blades *Conchocelis*	blades *Conchocelis*

[1] Some sporulation took place after 30 days, but spores were formed abundantly only after 65 days.
[2] Some sporulation took place after 22 days in the first generation, but spores were formed abundantly only after 51 days.

The growth responses of the blade phase to the five daylength and temperature regimes are presented in Figure 42. The production of the large pigmented α spores and the smaller colorless β spores by the blade phase are illustrated in Figures 43, 44. The *Conchocelis* phase again developed at 25 C, but this time under the 10-, 12- and 14-hr daylengths, in the third generation in each instance. In each daylength regime, the number of blades produced far outnumbered the number of *Conchocelis* plants. The amount of *Conchocelis* formed increased as

TABLE 10

Summary of the results of experiment 4B with *P. leucosticta* (Isolate 1)

	Incubator				
	1	2	3	4	5
Experimentation time (days)	65	100	86	79	86
Number of generations	1	2	3	3	4
Average generation time (days)	65[1]	50[2]	28.7	26.3	21.5
Spore types formed	α,β	α,β	α,β	α,β	α,β
Resultant progeny	blades	blades	blades *Conchocelis*	blades *Conchocelis*	blades *Conchocelis*

[1] Some sporulation took place after 30 days, but spores were formed abundantly only after 65 days.
[2] Some sporulation took place after 22 days in the first generation, but spores were formed abundantly only after 51 days.

1, formed at 25 C under a 12-hr daylength in incubator experiment 4B. One of the filaments is forming α spores.

FIG. 47. The elongated filaments of relatively small diameter of *Bangia fuscopurpurea*, isolate 3, formed at 25 C, under a 10-hr daylength in incubator experiment 4B. One of the filaments is forming β spores.

FIG. 48. The vegetative *Conchocelis* phase of *Bangia fuscopurpurea* at 25 C under a 14-hr daylength after 18 days in incubator experiment 4B.

FIG. 49. Blades of *Petalonia fascia* similar to those in nature, but of smaller dimensions, formed at 13 C under a 10-hr daylength regime in incubator experiment 4B. 78 days old, with plurilocular organs.

FIG. 50. A filamentous aggregation type of basal system which sprouted small blades at 25 C under a 10-hr daylength in incubator experiment 4B. 21 days old.

FIG. 51. A filamentous aggregation type of basal system which did not develop blades. It formed at 25 C under a 12-hr daylength in incubator experiment 4B. 42 days old.

the daylength increased: one plant was formed under the 10-hr daylength, a few plants under the 12-hr daylength and many plants under the 14-hr daylength regime.

Porphyra leucosticta, Conchocelis phase (Isolate 2): the inoculum consisted of the *Conchocelis* that had been produced by blades at 25 C under a 14-hr daylength earlier in the same experiment. At 25 C many conchosporangial branches developed under the 10- and 12-hr daylengths within 20 and 35 days, respectively, followed by the formation of a small number of blades 15 to 23 days later under the 10-hr daylength regime. At 25 C under a 14-hr daylength, a few inflated portions of the filaments one to two cells long developed, but extensive conchosporangial branches were absent. At 13 C the *Conchocelis* grew more slowly and was a paler reddish color than at 25 C. Occasional swollen cells developed under the 14-hr daylength, but with a 10-hr daylength the phase remained morphologically unchanged. The original inoculum gave a small number of new individuals, but these probably resulted from fragmentation. The experiment ran 56 days.

Porphyra leucostica, Conchocelis phase (Isolate 1): the inoculum consisted of the *Conchocelis* that had been produced by the blade phase at 25 C under a 14-hr daylength earlier in the same experiment. The results were similar to those of the preceding isolate, but no blades developed.

Bangia fuscopurpurea (Isolate 2): the results for the isolate in the experiment are summarized in Table 11. The algae grew much more rapidly and attained a larger size at 25 C than at 13 C, irrespective of the daylength regime employed. The filaments grew long and had a small diameter at 25 C, whereas at 13 C the filaments were stunted with a large diameter (Fig. 45). Alpha and β spores formed at 13 C, but at 25 C only α spores developed. In each generation the resultant progeny was the filamentous phase; the *Conchocelis* phase did not develop. The generation time was slower at 13 C than at 25 C. A daylength difference was apparent at the lower temperature, since reproduction proceeded more slowly under the 10-hr than under the 14-hr daylength, but at 25 C no significant daylength effects were apparent. The daylength difference at 13 C was probably a total light effect due to the alga receiving a greater amount of light

TABLE 11

Summary of the results of experiment 4B with *B. fuscopurpurea* (Isolate 2)

	Incubator				
	1	2	3	4	5
Experimentation time (days)	100[1]	72[1]	106	100	99
Number of generations	1	1	5	5	5
Average generation time (days)	78	50	21.2	20	19.8
Spore types formed	α,β	α,β	α	α	α
Resultant progeny	filaments	filaments	filaments	filaments	filaments

[1] The initial inoculum was discarded after 22 days since no growth took place, and new inoculum was placed in the experiment.

TABLE 12

Summary of the results of experiment 4B with *B. fuscopurpurea* (Isolate 1)

	Incubator				
	1	2	3	4	5
Experimentation time (days)	106[1,2]	106[1,3]	99	99	99
Number of generations	—	—	6	6	6
Average generation time (days)	—	—	16.5	16.5	16.5
Spore types formed	—	—	α	α	α
Progeny	—	—	filaments	filaments	filaments

[1] The initial inoculum was discarded after 22 days since no growth took place, and new inoculum was placed in the experiment.
[2] The plants were vegetative after 84 days, at the end of the experiment.
[3] Some sporulation occurred after 71 days but then ceased, and the plants were vegetative at the end of the experiment.

energy per day under the longer daylength than under the shorter daylength regime.

Bangia fuscopurpurea (Isolate 1): the results for the isolate are summarized in Table 12. The results were similar to isolate 1. The long filaments with a relatively small diameter that were formed at 25 C are illustrated in Figure 46. One of the filaments is producing α spores. However, the alga remained vegetative at 13 C under both daylength regimes, except for a little sporulation under the 14-hr daylength. Again the resultant progeny was the filamentous phase, with the absence of the *Conchocelis* phase.

Bangia fuscopurpurea (Isolate 3): the results for the isolate are summarized in Table 13. The alga again grew much more rapidly, attained a much larger size, and reproduced earlier at 25 C than at 13 C. The elongated filaments of relatively small diameter that were formed at 25 C are illustrated in Figure 47. One of the filaments is producing β spores. No daylength effects were apparent at the two temperatures. There was only a slight tendency for the filaments to thicken at 13 C. The main difference from the preceding two isolates was that α and β spores formed at 25 C as well as at 13 C.

Bangia fuscopurpurea, Conchocelis phase (Isolate 3): the phase grew well at 25 C under each daylength, but assumed a pale brownish coloration. The plants grew very slowly and were very pale brown at 13 C. The plant remained vegetative for the duration of the experiment, 98 days, under all conditions tested (Fig. 48).

TABLE 13

Summary of the results of experiment 4B with *B. fuscopurpurea* (Isolate 3)

	Incubator				
	1	2	3	4	5
Experimentation time (days)	43	43	56	48	56
Number of generations	1	1	4	4	4
Average generation time (days)	43	43	14	12	14
Spore types formed	α,β	α,β	$\alpha\beta$	α,β	α,β
Resultant progeny	filaments	filaments	filaments	filaments	filaments

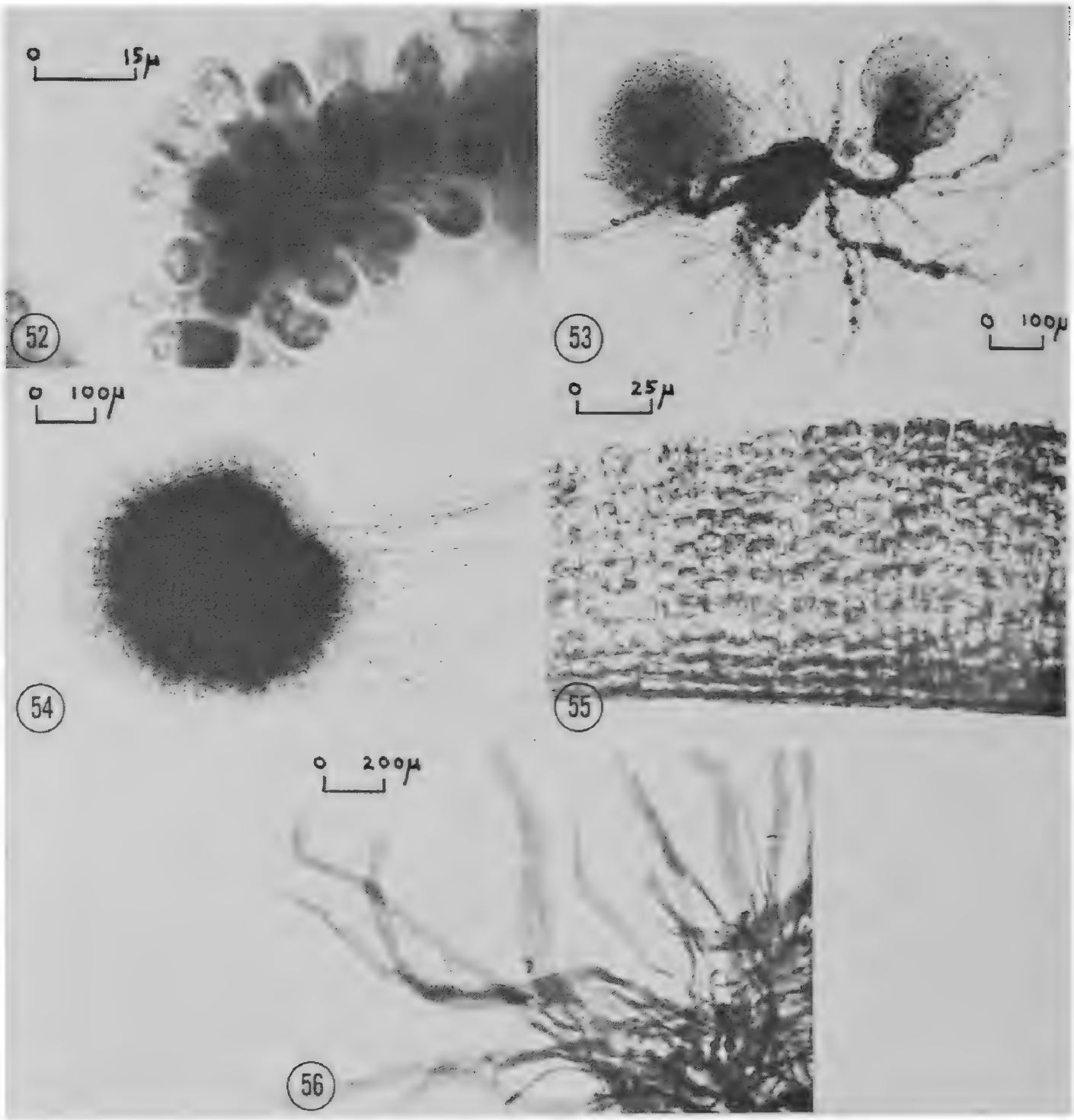


Fig. 52. A portion of the filamentous aggregation in Fig. 51, highly magnified.

Fig. 53. A basal system comprising both filamentous aggregation and discoidal portions which formed at 13 C under a 10-hr daylength in incubator experiment 4B. 28 days old.

Fig. 54. A discoidal basal system with colorless hairs, which developed at 13 C under a 14-hr daylength in incubator experiment 4B. 21 days old.

Fig. 55. A cross section of a discoidal basal system which developed at 25 C under a 10-hr daylength in incubator experiment 4B, after 55 days.

Fig. 56. A filamentous aggregation type of basal system which had developed at 25 C under a 14-hr daylength, sprouted blades after being transferred to 13 C under a 10-hr daylength regime in incubator experiment 4B, 14 days after the transfer.

However, a few swollen cells arranged in terminal, lateral and intercalary positions on the filaments, developed at 13 C under the 10-hr daylength regime.

Ectocarpus siliculosus: the alga attained a much larger size at 25 C than at 13 C. No growth differences were apparent under the different daylength regimes at each temperature. Plurilocular organs formed at 25 C, but the plants remained

vegetative at 13 C, after 21 days. Six days later the plants at 13 C had grown larger and had developed plurilocular organs but were still much smaller than were the plants at 25 C after 21 days.

Petalonia fascia: the experiment, which was repeated, gave similar results to those of experiment 4A (Figs. 49–56). Blades similar to those formed in nature but of smaller dimensions developed only at 13 C under the 10-hr daylength (Fig. 49). Large basal systems, which often gave rise to small blades, developed in the remaining regimes (Fig. 50). Again, none of the filamentous aggregations (Figs. 51, 52) or the discs became fertile. More extensive discs developed in this experiment, possibly because they were allowed to adhere to the bottom of the Petri dishes and were not disturbed on changing the medium (Figs. 53, 54). The base of the disc was usually composed of closely adhering filaments of quadrate cells which formed a compact layer of a few cells' thickness. Elongate cylindrical assimilatory filaments arose from the basal portion, up to 10–12 cells long (Fig. 55).

Filamentous aggregations, 72 days old, which had not developed blades, were transferred from the 25 C, 14-hr daylength regime to the 13 C, 10-hr daylength regime. Blades were produced by the filamentous aggregation within 7 days (Fig. 56).

Cross-Gradient Culture Apparatus Experiments

Experiment 1—Ectocarpus siliculosus

The light intensity values proceeding from the back to the front of the apparatus were: 35±5, 60±5, 110±5, 230±10, 380±10 and 510±10 ft c. The temperature value proceeding from the left to the right of the apparatus were: 10.5±1, 14.5±1, 19±1, 22.5±1, 26±1 and 29±1 C. The daylength was 14-hr.

Seawater of salinity 31 ppt, replenished every 7 days, was used for the medium. The experiment ran for 18 days.

The growth responses of the plants are given in Figure 57. Growth increased with an increase in light intensity and was optimal at approximately 230 ft c. The growth rate increased with an increase in temperature. The total amount of growth was maximal at 22.5 C and not at the highest temperature tested since reproduction, which also increased with temperature, reduced the growth rate to a greater extent at the higher than at the lower temperatures tested.

Plurilocular organs were the only type of reproductive structures formed. At the termination of the experiment, plurilocular organs had developed in all combinations of light intensity and temperature except 35 to 380 ft c at 10.5 C. Since the vegetative plants were healthy but growing slowly, they possibly might have reproduced at a later date. Figure 58 shows the development of plurilocular orgains after 14 days. They had formed under the high light intensity and temperature regimes, whilst the plants under the low light intensity and temperature regimes were still vegetative.

Experiment 2—Ceramium strictum

The light intensity values proceeding from the back to the front of the apparatus were: 35±5, 60±5, 110±5, 220±20, 360±20 and 500±20 ft c. The tempera-

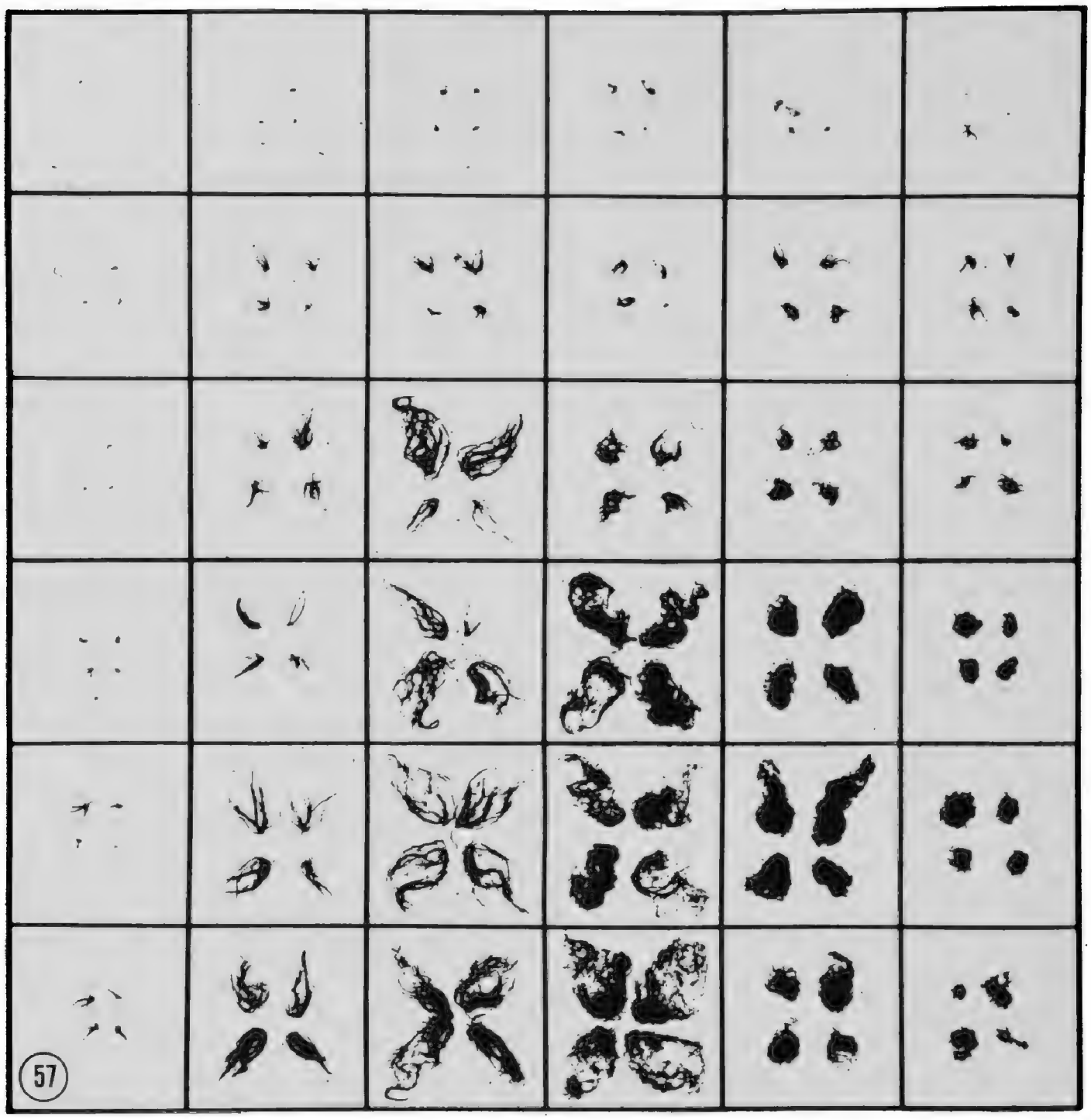


FIG. 57. The growth response of *Ectocarpus siliculosus* to varying light intensity and temperature regimes in the cross-gradient culture apparatus after 18 days. Experiment 1. The algae were mounted on a herbarium sheet prior to being photographed at the conclusion of the experiment. × 0.54.

ture values proceeding from the left to the right of the apparatus were: 10.5±1, 14.5±1, 19±1, 23±1, 26±1 and 30±1 C. The daylength regime was 14-hr.

Seawater of salinity 30–31 ppt, which was replenished after 8 days, was used for the medium. The experiment ran 15 days.

The growth responses of the alga are presented in Figure 59. Growth increased wit han increase in light intensity and temperature and was optimal at 500 ft c and 30 C, the maximum values tested for the two factors in the experiment. Growth occurred at 35 ft c, the lowest light intensity tested, but not at 10.5 C, the lowest temperature tested. The isolate remained vegetative under all regimes tested.

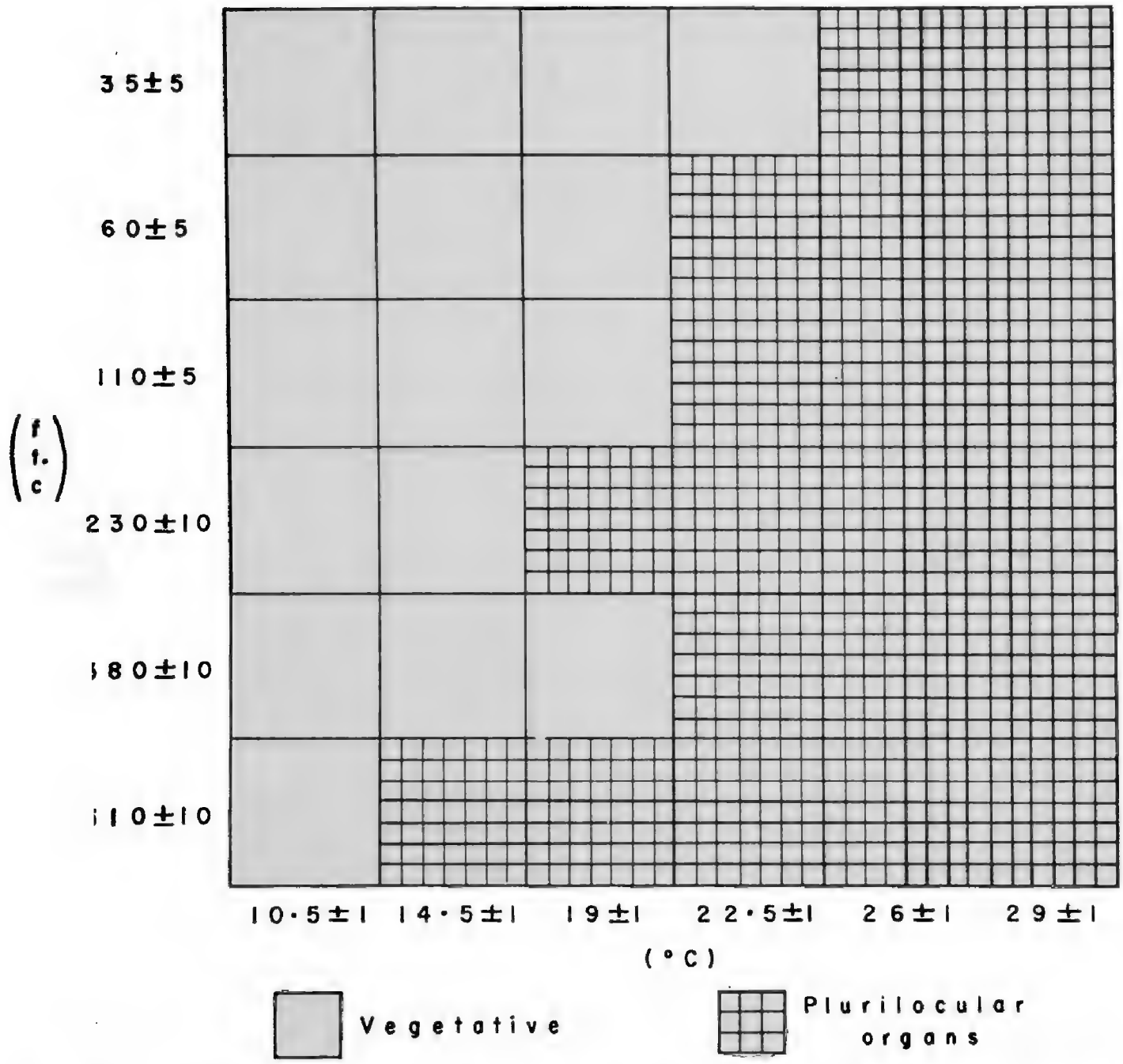


FIG. 58. Diagrammatic representation of the reproductive response of *Ectocarpus siliculosus* to varying light intensity and temperature regimes in the cross-gradient culture apparatus after 14 days. Experiment 1.

Experiment 3—Centroceras clavulatum

The light intensity, temperature and daylength regimes were the same as in the preceding experiment.

Seawater of salinity 31 ppt, which was replenished every 7 days, was used for the medium. The experiment ran 19 days.

The growth responses of the alga are given in Figure 60. The growth increased with increases in light intensity and temperature and was optimal at the higher values tested of the two parameters. Again, growth occurred at the lowest light intensity, but not at the lowest temperature tested. The isolate remained vegetative under all regimes tested.

Experiments 4–8—Polysiphonia boldii

The light intensity and temperature regimes employed are given diagrammatically in Figure 61. Short daylengths of 8-hr and long ones of 14-hr were

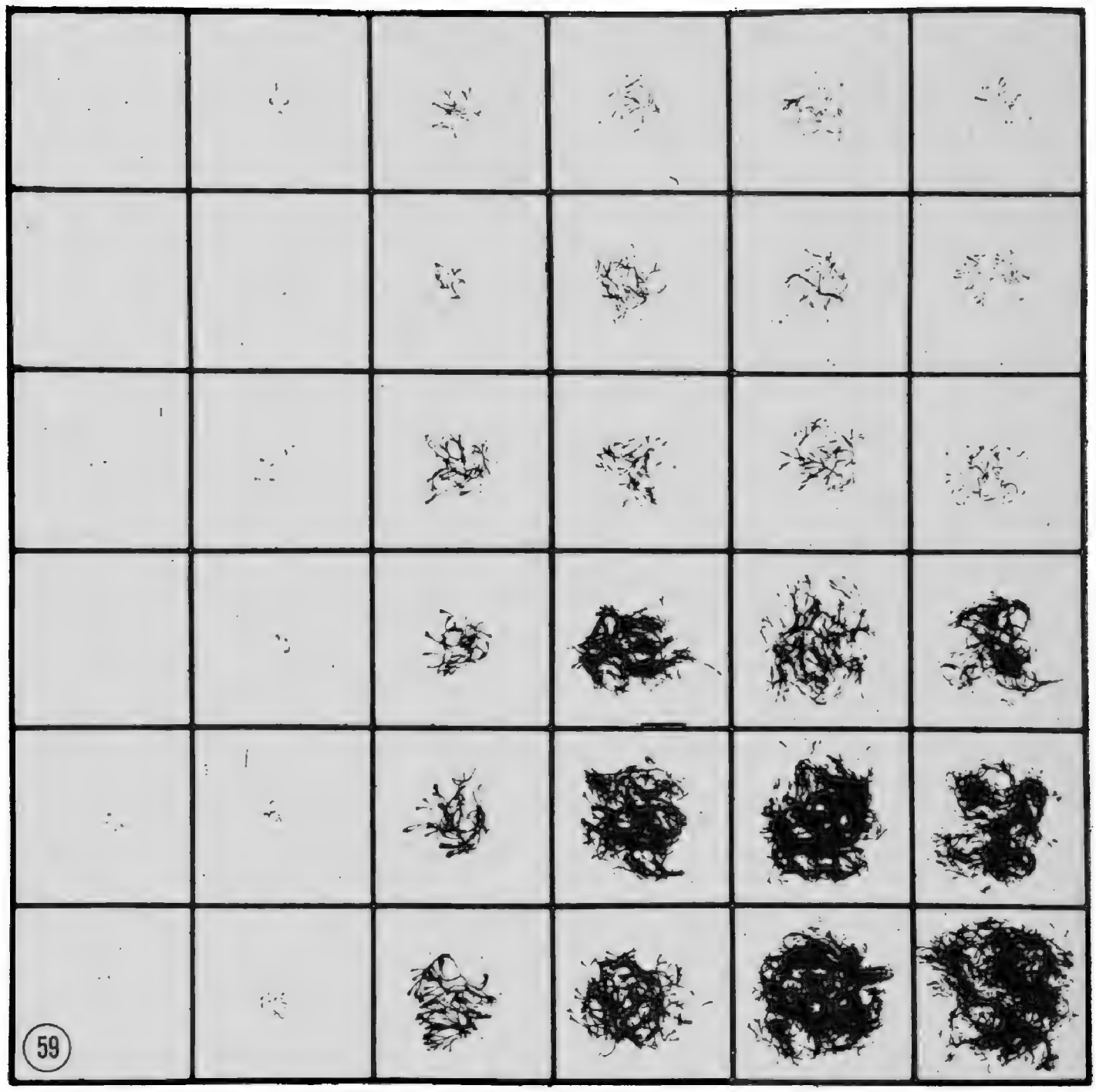


FIG. 59. The growth response of *Ceramium strictum* to varying light intensity and temperature regimes in the cross-gradient culture apparatus after 15 days. Experiment 2. The algae were mounted on a herbarium sheet prior to being photographed at the conclusion of the experiment. × 0.54.

utilized. It should be recalled at this point that in the cross-gradient culture experiments light intensity and temperature are the variables. Certain other data regarding the experiments are presented in Table 14.

Seawater of salinity 30–32 ppt was used for the medium. It was replenished initially after 14 days, and then after every 7 days.

The five experiments conducted with the species are summarized in Table 14. The experimentation time of the short daylength experiments was doubled relative to that of the long daylength experiments, so that the amount of light energy received for the duration of each experiment was approximately equal under the two daylength regimes. Observed differences could then be the attributed to daylength and not to total light effects.

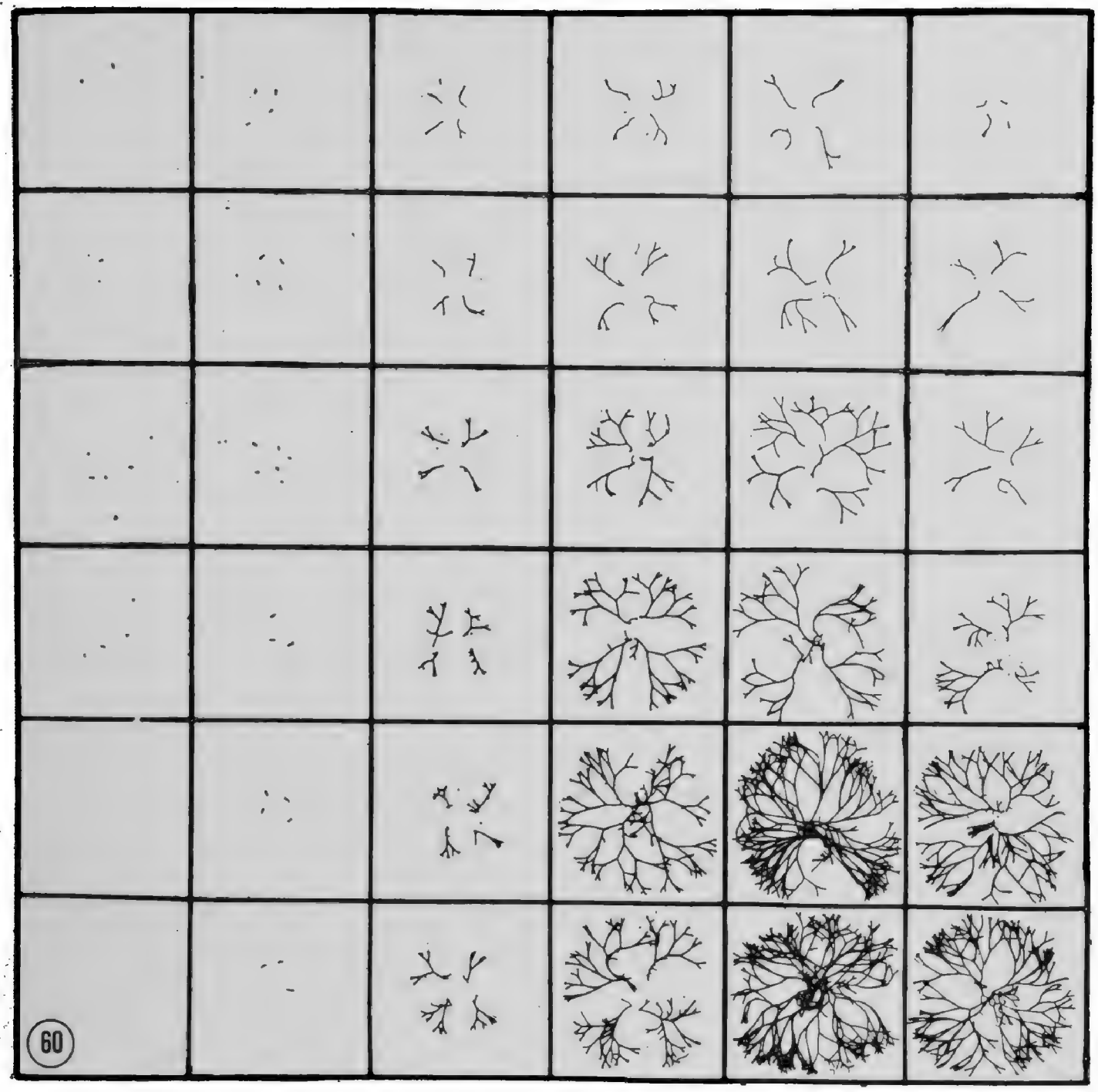


FIG. 60. The growth response of *Centroceras clavulatum* to varying light intensity and temperature regimes in the cross gradient culture apparatus after 19 days. Experiment 3. The algae were mounted on a herbarium sheet prior to being photographed at the conclusion of the experiment. × 0.54.

Growth responses of gametophytes and tetrasporophytes to many combinations of temperature and light intensity under short and long daylengths were similar (Figs. 62–65).

The amount of growth was independent of daylength. Similar amounts of growth were obtained under short and long daylengths, when the light energy received under the two daylength regimes was equalized by doubling the experimentation time of the short-day relative to that of the long-day experiments.

Little or no growth occurred at 10 C; growth increased with temperature from 14.5 C and was optimal at the higher temperatures tested.

Similarly, growth increased with an increase in light intensity and was optimal at the higher intensities tested.

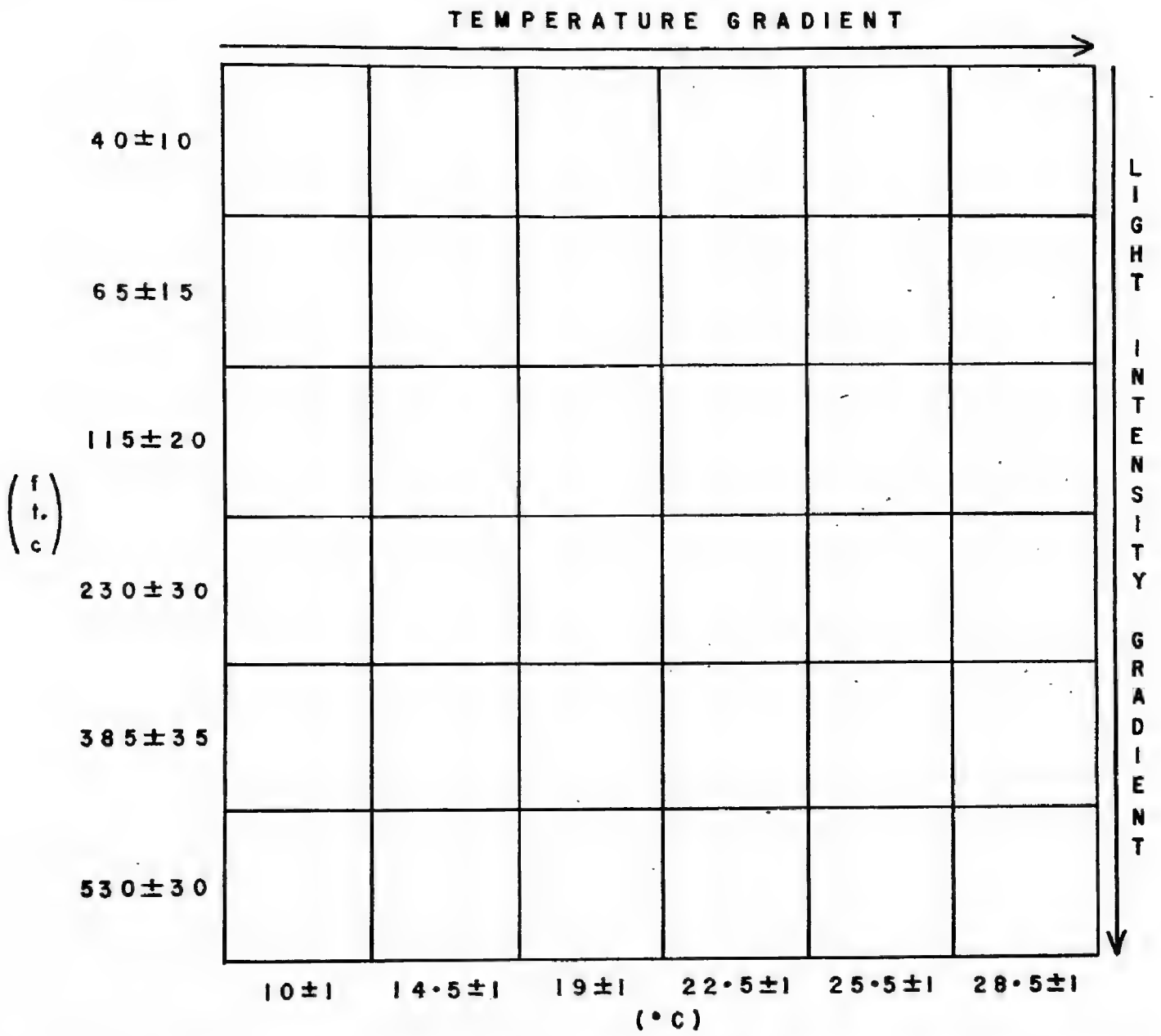


FIG. 61. A diagrammatic representation of the values for the temperature and light intensity gradients as used in cross gradient culture experiments 4–8 with *Polysiphonia boldii*.

TABLE 14

Additional parameters for the five cross-gradient culture apparatus experiments with *P. boldii*

Experiment Number	Inoculum	Daylength (hr)	Experimentation Time (days)	Illustration
4	Tetraspore germling	14	30	Fig. 66
5	Tetraspore germling	14	21	Figs. 62, 67
6	Tetraspore germling	8	43	Figs. 63, 68
7	Carpospore germling	14	21	Figs. 64, 69
8	Carpospore germling	8	42	Figs. 65, 70

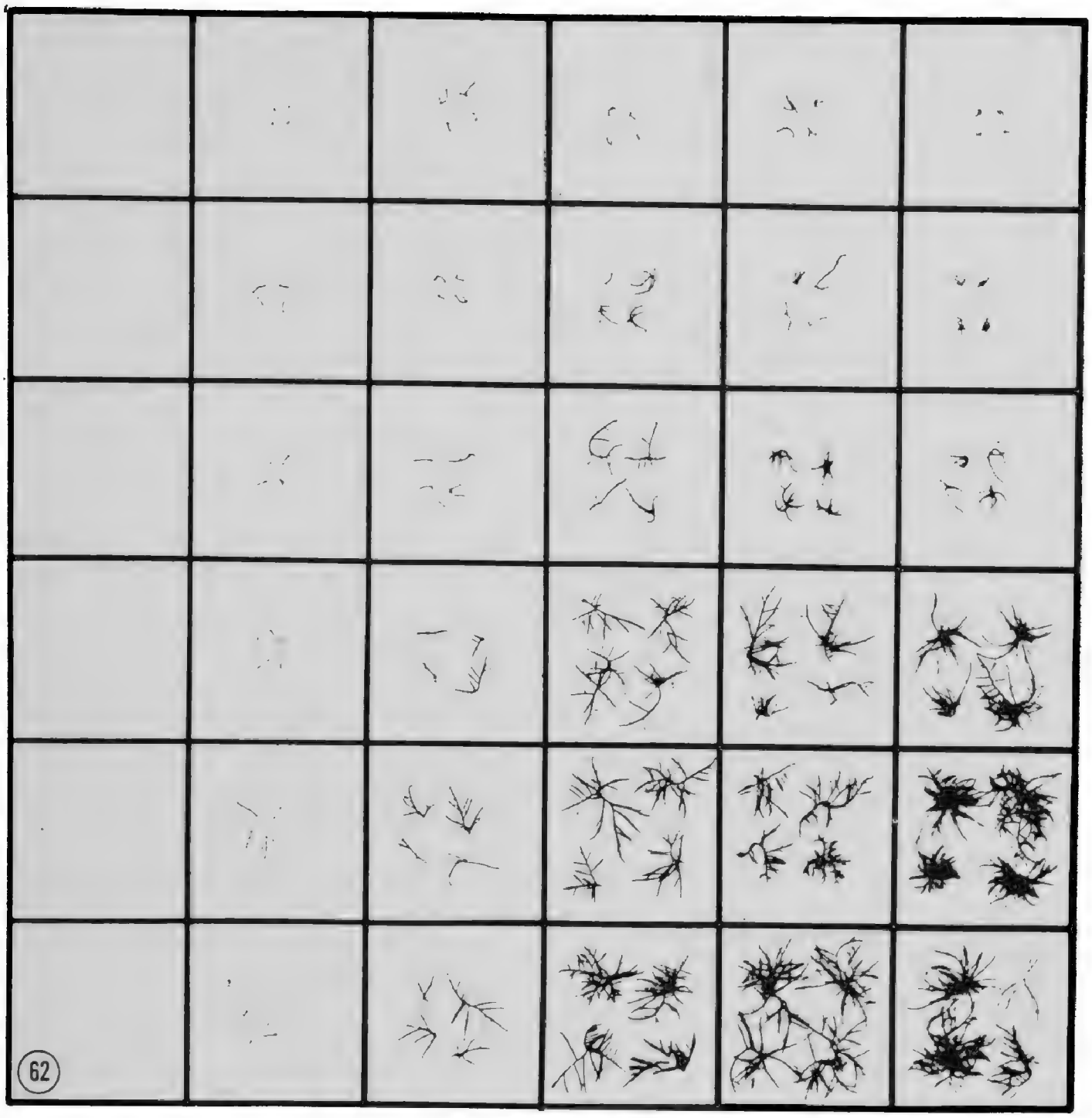


FIG. 62. The growth response of the gametophytic phase of *Polysiphonia boldii* under a day-length of 14-hr. Experiment 5.

An interaction between temperature and light intensity occurred, since at higher temperatures there was a high light intensity requirement for good growth in gametophytes under short days and in tetrasporophytes under long and short days (Figs. 63–65).

The formation of antheridia and carpogonia, carposporophytic development and carpospore release took place under both short and long daylengths, whereas tetrasporangial formation and tetraspore release occurred only under long day-lengths (Figs. 66–70).

No reproduction occurred at 10 C. Formation of antheridia and carpogonia, carposporophytic development and carpospore release required successively higher temperatures. Antheridia and carpogonia formed under all light intensi-ties, but carposporophytic development and carpospore release only took place

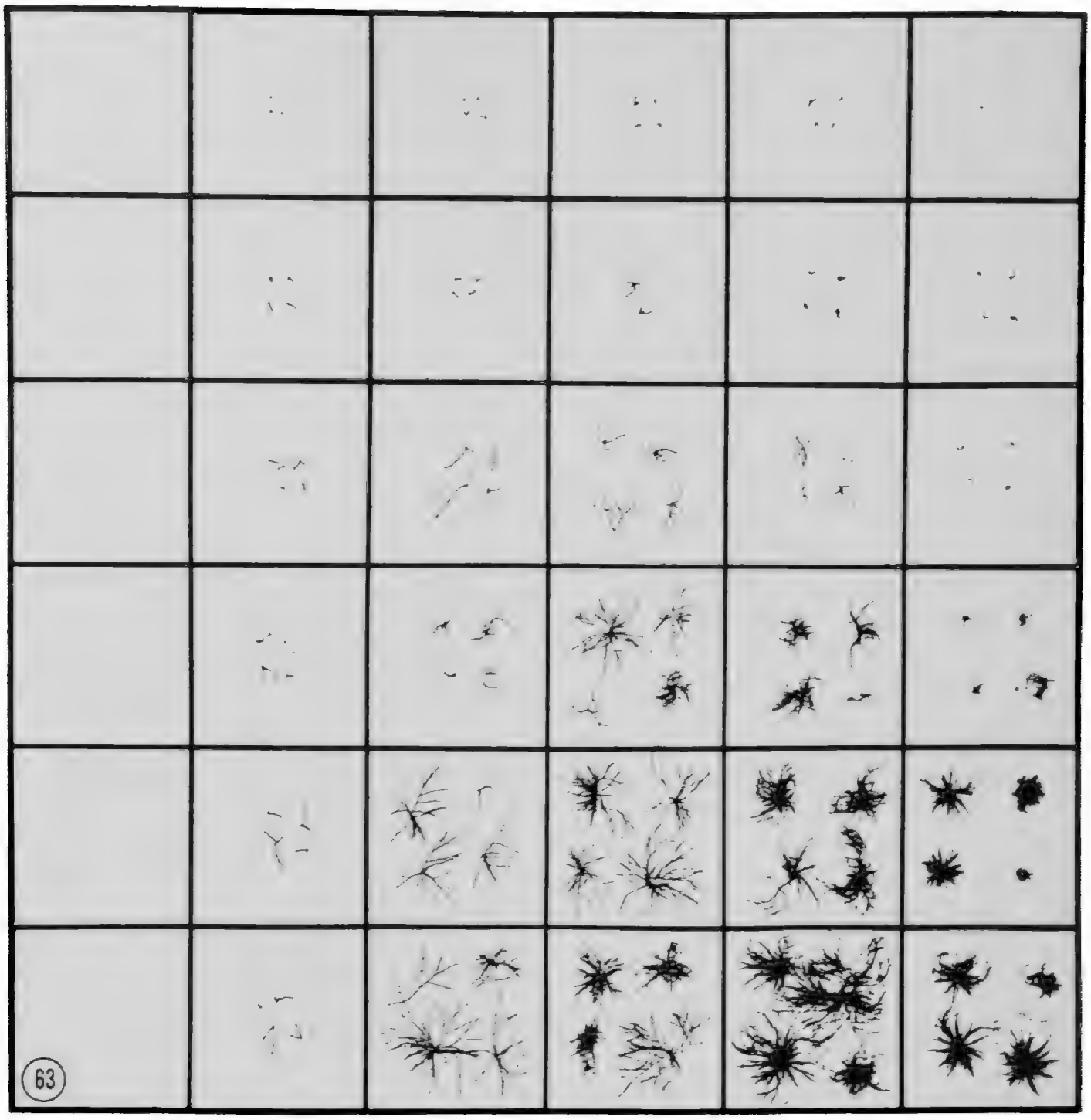


FIG. 63. The growth response of the gametophytic phase of *Polysiphonia boldii* under a day-length of 8-hr. Experiment 6.

at the higher light intensities. Under long daylengths, tetrasporangial formation and tetraspore release took place at the higher temperature and light intensity regimes.

Reproduction of gametophytic plants was apparently slower under short day-lengths than under long ones, in spite of the adjustment of the total light energy, as explained above.

An interaction between temperature and light intensity also took place with regard to reproduction in that at higher temperatures there was a high light intensity requirement.

Certain irregularities in the life-history were observed. Occasionally, tetra-spores gave rise to tetrasporophytes (Figs. 66, 67), and in one instance a carpo-spore produced a male gametophyte (Fig. 70).

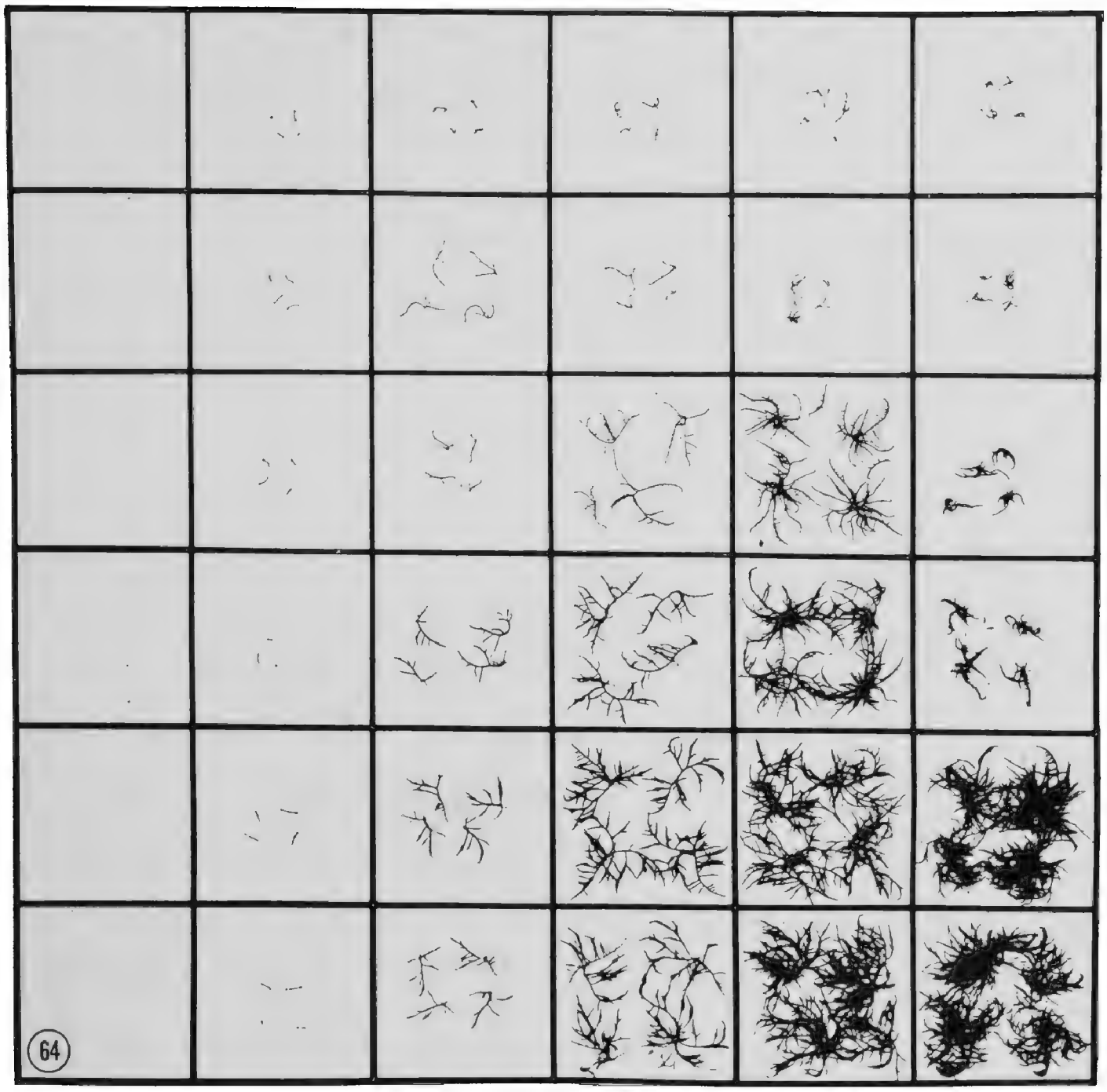


FIG. 64. The growth response of the tetrasporophytic phase of *Polysiphonia boldii* under a day-length of 14-hr. Experiment 7.

Life-History Observations in Culture of *Polysiphonia boldii*

Polysiphonia boldii was cultured through six successive generations between July 4, 1967 and September 14, 1968. The times taken in days for the completion of the various phases for the first four generations and are presented in Table 15.

The gametophytic and tetrasporophytic phases took approximately 1 month to develop to maturity from tetraspore and carpospore germlings, respectively, while carposporophytic development and carpospore release took about 2 weeks. The completion of one generation of the life-history took from 2–3 months. The time required for the completion of a generation in culture decreased with succeeding generations, possibly because the isolate was becoming better adapted to culture conditions.

The alga was largely dioecious, but plants bearing both antheridia and carpogonia were not uncommon.

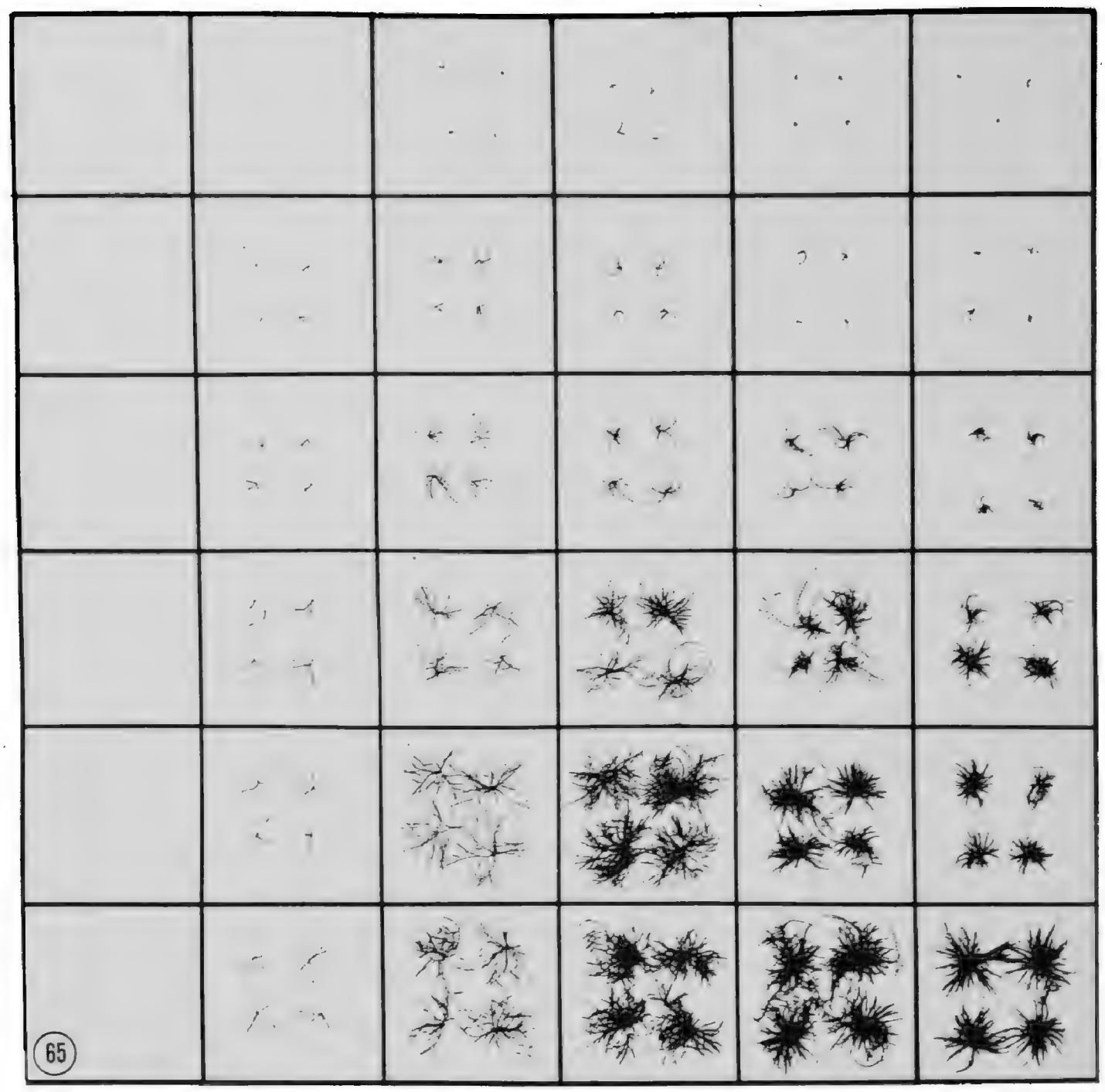
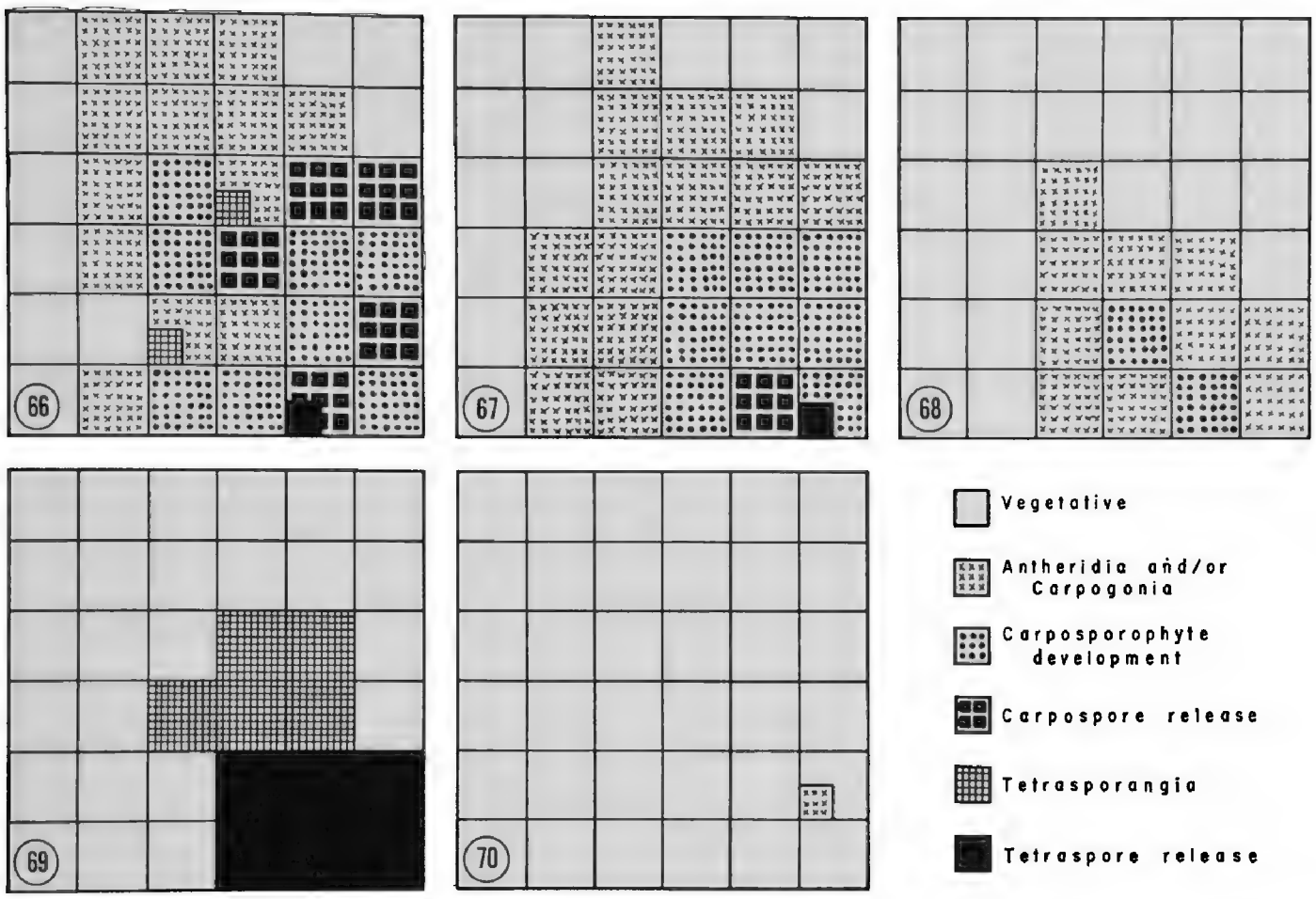


FIG. 65. The growth response of the tetrasporophytic phase of *Polysiphonia boldii* under a day-length of 8-hr. Experiment 8.

TABLE 15

Time (in days) required for the completion of the various phases that comprise the life-history of *P. boldii*

Generation	Tetraspore to antheridia and carpogonia	Antheridia and carpogonia to carpospores	Carpospores to tetraspores	Complete generation (tetraspore to tetraspore)
1	49	13	43	105
2	35	13	41	89
3	26	20	27	73
4	20	16	26	62
Average Time	32	15	34	82



FIGS. 66–70. Diagrammatic representation of the reproductive responses of *Polysiphonia boldii* to varying light intensity and temperature regimes in the cross-gradient culture apparatus. Experiments 4–8.

FIG. 66. The reproductive response of the gametophytic phase under a daylength of 14-hr. Experiment 4.

FIG. 67. The reproductive response of the gametophytic phase under a daylength of 14-hr. Experiment 5.

FIG. 68. The reproductive response of the gametophytic phase under a daylength of 8-hr. Experiment 6.

FIG. 69. The reproductive response of the tetrasporophytic phase under a daylength of 14-hr. Experiment 7.

FIG. 70. The reproductive response of the tetrasporophytic phase under a daylength of 8-hr. Experiment 8.

The life-history of *P. boldii* in culture comprised the sequence of events that is generally held to constitute the typical life-history of the Florideophycidae (Edwards 1968) and was similar in this respect to *Callithamnion byssoides* (Edwards 1969). Certain irregularities were observed, but these appeared to play a much less significant part of the life-history than in other red algae (West and Norris 1966; Hassinger-Huizinga 1952).

SUMMARY OF THE RESULTS OF THE CULTURAL STUDIES

The growth and reproductive responses of the several algae investigated to variations of light-intensity, daylength and temperature, as revealed by the incubator and cross-gradient culture apparatus experiments, are summarized below.

Phaeophycophyta

Giffordia mitchelliae

Growth was saturated at a light intensity of 140±20 ft c. Daylength variations had no apparent effect on the growth of this species. The alga died at 9.5±0.5 C; growth proceeded slowly at 14.5±0.5 C and then increased with temperature until growth was optimal at 24.5±0.5 C. The growth was less than optimal at 29.5±0.5 C.

Plurilocular organs developed in all conditions in which growth took place.

Ectocarpus siliculosus

Growth was saturated at 230±10 ft c. Daylength had no apparent effect on the growth of the alga. The growth and reproductive rates increased with an increase in temperature. The latter reduced the amount of growth to a greater degree at the higher than at the lower temperatures tested so that the total growth maximum occurred at approximately 22.5±0.5 C.

Plurilocular organs were formed in practically all conditions in which growth proceeded.

Petalonia fascia

Blades resembling those found in nature developed in culture only at 13 C under a short daylength of 8- or 10-hr. At 13 C under a 14- or 16-hr daylength or at 25 C with daylengths of 8–16-hr, large basal systems developed, which occasionally gave rise to small blades.

Plurilocular organs, produced on the blades, were the only type of reproductive organ observed, developing in all regimes tested.

Rhodophycophyta

Bangiophycidae

Erythrocladia subintegra

Growth was light-saturated at 50±5 ft c. Daylength variations had no apparent effect on growth, which increased with increasing temperature and was optimal at 19±1 C.

Monospores were produced in all conditions in which growth proceeded.

Goniotrichum alsidii

The growth of the species was light-saturated at 50±5 ft c. Good growth resulted in daylength regimes of 12–14 hr, but the growth rate was less in the 8-hr daylength regime. Growth did not occur at 9.5 and 14.5±0.5 C, was slow at 19±1 C and was optimal at 24.5 and 29.5±0.5 C. The plants became pluriseriate under all conditions and developed monospores.

Bangia fuscopurpurea

Growth was light-saturated at 140±20 ft c. Daylength variation had little apparent effect on growth except for a slight depression of the growth rate in continuous light. The alga died at 9.5±0.5 C; growth proceeded slowly at 13±1 C

and then increased with increasing temperature to reach a maximum at 24.5 and 29.5±0.5 C. The filamentous phase consisted of long filaments of relatively small diameter, comparable to plants found in nature, at the higher temperatures tested; but at lower temperatures, the filaments tended to be shorter and thicker.

Reproduction generally accompanied growth. Isolates 1 and 2 produced mainly α spores, with the occasional production of β spores taking place at 13±1 C. Isolate 3, however, formed an abundance of α and β spores under all conditions in which the alga was cultured.

The alternative phase in the life-history, *Conchocelis*, was not produced by any of the isolates during the experiments. It arose from isolate 3 when the filamentous phase from nature was grown in a short daylength of 14-hr with 205±55 ft c illumination at 25 C. The filamentous phase was largely repeated under a 10-hr daylength with the same lighting and temperature conditions. The reconstitution of the filamentous phase from the *Conchocelis* was not effected.

Porphyra leucosticta

Growth of the blade phase was saturated at 140±20 ft c. Daylength appeared to regulate the interconversion of the bade and *Conchocelis* phases of the life-history. The production of *Conchocelis* by the blade phase took place in daylengths of 10-hr or longer and the amount of *Conchocelis* formed increased with an increase in daylength. The plants grew slowly at the lower temperatures tested, assumed a healthy pinkish-red coloration and attained a relatively large size before reproducing. At the higher temperatures, the blades grew much faster, assumed an unhealthy brownish-red coloration and reproduced when still quite small. Alpha and β spores were generally produced in all regimes tested.

The growth of the *Conchocelis* phase was apparently similar in all light intensity regimes tested. Short daylengths appeared to cause the *Conchocelis* phase to produce blades, while the *Conchocelis* phase remained vegetative under long daylengths. The *Conchocelis* phase grew well between 14.5 and 24.5±0.5 C; but at the lower and higher temperatures investigated, growth was much less.

Conchosporangial branches, produced largely under short daylengths, were the only type of reproductive structures observed in *Conchocelis*.

Florideophycidae

Acrochaetium crassipes

Growth was light-saturated at 140±20 ft c. Good growth occurred under daylengths of 12–20-hr, but growth was below optimal under an 8-hr daylength and in continuous light. The alga died at 9.5±0.5 C; growth proceeded slowly at 14.5±0.5 C and then increased with an increase in temperature until optimal growth was attained at 24.5 and 29.5±0.5 C. Monospore formation took place under all conditions.

Acrochaetium flexuosum

Growth was saturated at 140 ± 20 ft c. Good growth occurred in daylengths of 12- to 24-hr, but growth was slow under an 8-hr daylength. The alga died at

9.5±0.5 C; growth proceeded slowly at 14.5±0.5 C and then increased with temperature to a maximum at 29.5±0.5 C. Monospores formed in all culture regimes.

Ceramium strictum

Growth increased with an increase in light intensity and was optimal at a little over 100 ft c. Optimal growth occurred in daylength regimes of 16- and 20-hr; in daylength of 8-, 12- and 24-hr, growth was less than optimal. The plants died at 9.5±0.5 C; growth began at 14.5±1 C and increased with temperature and was optimal at 24.5±0.5 C and 30±1 C. The alga remained vegetative under all regimes tested.

Centroceras clavulatum

Growth increased with an increase in light intensity and was optimal at approximately 400 ft c. Good growth resulted in daylength regimes of 8–20-hr, but growth was slightly less than optimal in continuous light. In incubator experiment 3 the alga died at 9.5 and 14.5±0.5 C; growth increased with temperature from 19 ± 1 C, but the plants were stunted at 29.5 ± 0.5 C. The results with the isolate in the cross-gradient experiment 3 were at variance; the growth again increased with temperature, but the alga grew slowly at 14.5±1 C and demonstrated optimal growth at 30±1 C. The alga remained vegetative under all regimes tested.

Polysiphonia boldii

Growth increased with an increase in light-intensity and was optimal at 140± 20 ft c; the growth was less than optimal at the higher intensity values tested. Similar amounts of growth occurred under all daylength regimes. The alga died at 9.5±1 C; growth proceeded slowly at 14.5±1 C and then increased with temperature until optimal growth was attained at 24.5 and 29.5±1 C.

Light intensity had little apparent effect on the induction of reproductive organs. Daylength had a profound effect on reproduction; the formation of antheridia and carpogonia, carposporophytic development and carpospore release took place independently of daylength (8–24-hr); but tetrasporangia did not form in short days of 8-hr or in continuous light. The alga reproduced from 14.5 to 28.5±1 C; but at the highest temperature tested, antheridia and carpogonia developed while tetrasporangia were not produced.

DISCUSSION AND CONCLUSIONS

The growth and reproductive responses of twelve benthic marine algae in culture to variations of light intensity, daylength and temperature have been investigated.

Variations in light intensity in the laboratory had a relatively small effect on the growth and reproduction of the algae studied. The growth of the algae was saturated at low light intensities and although reproduction took place faster as the intensity was increased, it generally occurred over the entire range of intensities tested. Boalch (1961) cultured *Ectocarpus confervoides* (=*E. siliculosus*) in varying light intensities and found that this had little effect on the alga; he concluded that growth was probably saturated at low intensities. Edwards

(in press) demonstrated that the growth of *Callithamnion byssoides* was saturated at low light intensities; the maturation of the gametophytic phase took place over a wide range of intensities, but the development of tetrasporangia occurred only in a narrow range. Light intensity was shown by West (1967, 1968, 1969) to have little effect on reproduction in *Rhodochorton purpureum* and *Acrochaetium pectinatum*. Knaggs (1966a, 1966b), however, induced tetrasporangial formation in *Rhodochorton purpureum* by exposing the plants to a higher light intensity than that to which they had become adapted. Plants cultured at 5 C under an 8-hr daylength and initially subjected to 500 ergs/cm^2-sec or 4,000 ergs/cm^2-sec and then transferred to 4,000 and 8,000 ergs/cm^2-sec, respectively, and those transferred from 4,000 to 500 ergs/cm^2-sec and then back again to 4,000 ergs/cm^2-sec produced tetrasporangia, while plants transferred from 4,000 to 500 ergs/cm^2-sec or held under a constant light intensity of 4,000 ergs/cm^2-sec remained vegetative.

The red algae assumed a brownish color in culture at light intensities of over 500 ft c, an indication that they were possibly intolerant of elevated light intensity. An adverse effect of relatively low light intensities in culture was also reported by West (1968) in *Acrochaetium pectinatum* and by Knaggs (1966a) in *Rhodochorton purpureum*.

Many algae, particularly those from the sublittoral zone, may be exposed only to low light intensity in nature and one would thus expect their growth to be saturated at low light intensities in culture. *Centroceras clavulatum*, however, was also adversely affected by the highest light intensity values tested in culture, but in nature is exposed to high intensities of light, at least periodically, since the alga grows in the upper eulittoral zone. The alga may tolerate high intensities for short periods under natural conditions, but may be adversely affected by prolonged exposure to elevated light intensities. Perhaps the poor mimicking of natural light conditions in the laboratory contributes towards the adverse effect of relatively low light intensities on the algae. In the laboratory, algae are subjected to a square, rather than to a natural light curve; a sudden change takes place twice daily between darkness and the constant light intensity value under investigation when the lights go on and off. The spectral emission of the light source, which is also relatively constant, differs from the natural spectrum and this may also contribute towards the harmful effects of the higher light intensities studied.

However, since changes in the growth and reproductive responses of algae similar to those observed in nature can be induced in culture under constant low light intensity by manipulating factors other than the former, seasonal variations in light intensity may not be of primary importance in determining the seasonal alterations of algal floras in nature.

The effects of variation in daylength on the algae differed depending on the species. Those species studied whose life-history consists of a single phase or of two morphologically similar phases, demonstrated good growth in the laboratory throughout the range of daylengths encountered in the natural habitat of the species concerned. Daylengths shorter or longer than the seasonal range in nature often depressed the growth rate of the species; *e.g.*, the growth rate of *Gonio-*

trichum alsidii was reduced under an 8-hr daylength, that of *Acrochaetium crassipes* under an 8-hr daylength and in continuous light and that of *Centroceras clavulatum* in continuous light. However, the growth rates of *Giffordia mitchelliae* and *Polysiphonia boldii* were unaffected by daylength variations. The reproduction of those algae that consist of a single phase took place asexually by monospores and the process was unaffected by daylength; *e.g.*, *Erythrocladia subintegra* and *Acrochaetium flexuosum*. However, the reproduction of *Polysiphonia boldii*, which consists of two morphologically similar phases, was influenced by daylengths, since tetrasporangia would not develop in short daylengths of 8-hr. Daylength had a profound effect on taxa whose life-history consists of two morphologically dissimilar phases and was of primary importance in effecting an alteration between the two phases in culture; *e.g.*, *Petalonia fascia* and *Porphyra leucosticta*.

Temperature variations had a pronounced effect on the growth and reproduction of the algae studied. The algae were generally adapted to the temperature range of their natural environment; the majority of the species died when subjected to a temperature of 9.5 ± 0.5 C which is approximately 3 C lower than the minimum mean monthly water temperature for Port Aransas. *Ectocarpus siliculosus* and *Porphyra leucosticta* grew slowly at 9.5 ± 0.5 C, but this is not surprising since they are of north Atlantic affinity where winter temperatures fall far below this value.

The growth rate of all the species investigated increased with an increase in temperature. Species of tropical Caribbean affinity (*e.g.*, *Centroceras clavulatum* and *Ceramium strictum*) demonstrated maximum growth rate and maximum amount of growth at the higher temperatures tested. The maximum amount of growth of *Ectocarpus siliculosus* and *Porphyra leucosticta*, species of cool temperate north Atlantic affinity, occurred at the lower and intermediate temperatures tested, respectively, since the reproductive rate which increased with an increase in temperature limited the amount of growth to a greater degree at the higher than at the lower temperatures tested.

An attempt has been made to explain the observed seasonal distribution of the growth and reproduction of each species in nature on the basis of the information obtained in culture.

The growth of *Giffordia mitchelliae* in culture proceeded in the laboratory over the annual range of water temperatures at Port Aransas and attained maximal values in water temperatures which corresponded to those of late spring and early summer in nature. The growth of the species in the study area did not follow the seasonal water temperature cycle, but was irregular. Factors other than those investigated during the study appear to be of primary importance in the growth of the species in nature. Plurilocular organs were the only type of reproductive structure observed in culture. In nature the alga was collected almost invariably with plurilocular organs, but on two occasions unilocular organs were observed. The life-history of the entity in the study area possibly consists largely of an asexual repetition of a single phase by swarmers from plurilocular organs.

Cultural studies indicated that the optimal temperature for growth of *Ectocarpus siliculosus* in culture was higher than the sea temperature when the alga was

present in the study area. The optimal growth temperature in culture coincided with the water temperature in nature when the species was beginning to disappear. Since daylength had no apparent effect on growth, an unknown factor is implicated as a causal factor in the seasonal distribution of *E. siliculosus*. Nutrition may be involved. A high nutrient level of the cultural medium was employed, but in nature the low nitrate and phosphate concentrations of the sea in summer (Copeland *pers. comm.*) may be insufficient to support growth despite the elevated water temperature. The life-history of the species varies in different parts of the world (review in Russell 1966). Papenfuss (1935) described the life-history of the species in the Woods Hole area, Massachusetts, as an isomorphic (tending to dimorphic) diplobiont. Diploid asexual plants bore plurilocular and unilocular organs. The swarmers from the former type of reproductive organ repeated the phase, but meiosis occurred in the unilocular organs to give haploid swarmers, which developed into dioecious sexual plants. The swarmers from the plurilocular gametangia of the sexual plants fused and the zygotes reconstituted the diploid asexual phase. Plants bearing plurilocular organs were the only type of individuals observed by the writer during the study in nature and in culture, so that in the Port Aransas area the alga possibly consists of a single phase which reproduced itself asexually by swarmers from plurilocular organs.

Alternate phases in the life-history of *Petalonia fascia* have been described by previous investigators from different parts of the world. Dangeard (1963), in France, obtained a filamentous phase from the swarmers of the plurilocular organs of the blade phase, with discoid or glomerulate regions, very similar to the one obtained in the present study. The filamentous stage also sprouted the blade phase, but the sessile plurilocular organs, which were occasionally observed on the filamentous phase, were absent from the Texas isolate of the species. Nakamura (1965), in Japan, obtained *Petalonia fascia* from the swarmers of the unilocular organs of *Ralfsia*-like plants. The most extensive study to date was conducted by Wynne (1969), who again found two dissimilar morphological phases in the life-history of the species from the Pacific coast of North America; the familiar blade-like thallus of *Petalonia fascia* and an inconspicuous, crustose *Ralfsia*-like phase bearing unilocular organs. He reported an absence of a strict heteromorphic alternation of generations but found that a combination of cool temperatures and short daylengths favored the development of the blade phase and warm temperatures and long daylengths favored the development of the crustose stage. He considered the basal system of the blade phase and the discoid phase bearing unilocular organs to be homologous structures since discs were occasionally found bearing both unilocular organs and blades. Since evidence that the two phases were linked by a sexual cycle was absent, he concluded that the species has a "flexible asexual life-history involving two morphologically different stages whose expressions are governed by environmental conditions rather than by an obligate alternation of ploidy level." The present study confirms the work of Wynne (1969) in that blades comparable to the ones found in nature developed only under a *combination* of cool water temperatures and short daylengths. It contrasts with his work in that the alternate phase obtained in the present study was largely filamentous in nature and was only occasionally dis-

coid and it did not become fertile. Cultural studies thus indicate that the species passes the warmer months of the year as a filamentous or discoid alternative phase, but none has been collected in nature.

The seasonal occurrence of *Erythrocladia subintegra* appears to be determined solely by the water temperature. The alga, which occurred throughout the year in the study area, grows in culture over the range of water temperatures found at Port Aransas and shows optimal growth in the higher temperatures tested.

The seasonal distribution of *Goniotrichum alsidii* similarly appears to be controlled by water temperatures. The alga was not observed during the cooler months in the study area. Growth of the alga in culture began at 19±1 C and showed optimal values at the higher temperatures tested. One apparent anomaly between observations in nature and in culture was the occurrence of a pluriseriate condition. The condition was observed on a single occasion in nature, but was almost invariably present in culture.

Drew (1952) first obtained a filamentous *Conchocelis* in *Bangia fuscopurpurea* but she did not obtain the complete life-history in culture. Richardson and Dixon (1968) completed the life-history of the species in culture and implicated daylength as a controlling factor. The *Conchocelis* phase arose from *Bangia* in all daylengths longer than 12-hr, whilst daylengths of less than 12-hr induced the formation of the *Bangia* phase from the *Conchocelis*. The *Conchocelis* phase produced conchosporangial branches from 5–15 C but not at 20 C, so that the life-history could not be completed at the higher temperature. Sommerfeld (*pers. comm.*) also studied the life history of *Bangia fuscopurpurea* in culture, but temperature appeared to be an inductive factor. The *Bangia* phase gave rise to more filaments at 22 C or above, to filaments and *Conchocelis* at 15 C and to *Conchocelis* at 9 C. The life-history of the species could not be induced in culture during the present study. The *Conchocelis* phase arose once in culture, when the filamentous phase of isolate 3 was first cultured under a long daylength of 14-hr; under a 10-hr daylength the filamentous phase was largely repeated. Daylength thus appears to be involved in the conversion of the filamentous to the *Conchocelis* phase. The filamentous phase grew best in culture at the higher temperatures tested when the filaments had a morphology comparable to those found in nature; at the lower temperatures tested the growth rate was extremely slow and the filaments were stunted in form. The temperature response of the alga in culture is difficult to understand, since the species shows maximal development in nature during winter and early spring. The species occurs in the littoral fringe on the southwest jetty at Port Aransas, where it is not submerged but is kept moist by splash and spray. Since it is exposed to the air it is subjected more to air than to water temperatures. The higher average air temperature to which it is exposed may be responsible for its faster growth rate at elevated water temperatures in culture. The anomalous cultural growth response may be due in part to the alga being submerged in culture. The absence of the species on the jetties at Cape Lookout, as reported by Williams (1949), is probably due to the submersion of the jetty at high tide.

An alternative microscopic phase in the life-history of *Porphyra leucosticta* has been reported by several investigators. Kornmann (1961) obtained the com-

plete life-history in culture in Helgoland, but not under controlled environmental conditions. Conway (1961) reported a *Conchocelis* phase in the life-history of the species from Scotland, but gave no details. Sommerfeld (*pers. comm.*) completed the life-history of the species from Woods Hole, Massachussetts in culture and implicated variations in daylength as a controlling factor. The blade phase gave rise to blades and the *Conchocelis* phase under a 12-hr daylength; the *Conchocelis* phase reconstituted the blade phase under daylength regimes of 6- and 12-hr. Iwasaki (1961) cultured a second species, *Porphyra tenera*. He found that both phases in the life-history grew over a temperature range of 7–21 C in culture, but that the interconversion of the two phases was determined by daylength variations. Short daylengths of 8–12-hr induced the production of blades by the *Conchocelis* phase, whereas a daylength of 13-hr inhibited the growth of the blades and induced them to form the *Conchocelis* phase. The life-history of *P. leucosticta* was completed in culture during the present study. Daylength appeared to regulate the interconversion of the blade and *Conchocelis* phases of the life-history, as had been demonstrated by previous workers. The blade phase gave rise to *Conchocelis* in daylengths of 10-hr or longer and the amount of *Conchocelis* formed increased as the daylength lengthened; short daylengths appeared to cause the *Conchocelis* phase to produce blades, whilst under long daylength regimes the *Conchocelis* remained vegetative. Temperature was also involved as a controlling factor in the life-history. Relatively large blades comparable to those found in nature occurred only at the lower temperatures; at the higher temperatures tested the blades had a much faster growth rate and reproduced when quite young, so that a large size was not attained.

Acrochaetium crassipes and *A. flexuosum* occurred throughout the year in the study area. There is a general correlation between the occurrence of the algae in nature and the water temperature obtained, since the algae grew in culture over the range of water temperatures found at Port Aransas with maximum growth taking place at the higher temperatures for both species.

The seasonal distribution of *Ceramium strictum* may largely be interpreted on the basis of temperature. The occurrence of the alga in abundance in nature during the warmer months of the year correlates well with the growth of the species in culture, where optimal growth takes place at the higher temperatures tested.

The seasonal occurrence of *Centroceras clavulatum* similarly appears to be determined by the water temperature, with elevated water temperatures favoring good growth.

A correlation with regard to temperature may be drawn between the growth of *Polysiphonia boldii* in culture and in nature at Port Aransas. The growth of the alga in culture increased with an increase in temperature and was optimal at the higher temperatures tested. The alga was present throughout the year in the study area, but was apparently more common during the warmer months of the year. The alga reproduced in culture over practically the entire range of temperatures found in nature in the study area, but at the highest temperature tested antheridia and carpogonia developed while tetrasporangia were not formed. The reproduction of the species was apparently unaffected in culture by the range of daylengths found in the Port Aransas area. However, tetrasporangia did not de-

velop under a short daylength of 8-hr or in continuous light, two artificial daylength regimes for the area under consideration. Daylength was also shown by West (1967, 1968) to be a controlling factor in the reproduction of *Rhodochorton purpureum* and *Acrochaetium pectinatum*. Daylength again had no effect on the formation of sex organs in these two genera, but tetrasporangia developed only under short daylengths. The production of tetrasporangia in culture by *R. purpureum* in short daylengths only, correlates well with field data, which indicate that the species develops tetrasporangia in nature in winter.

The present study supports the contention of a majority of previous investigators, based on field studies alone, that temperature is of primary importance in the seasonal distribution of algae in nature. Seasonal variations in daylength, acting alone or in conjunction with temperature variations, have in addition been implicated as a causal factor in the seasonal alteration of algal floras. Seasonal variations in light intensity, which were held by Conover (1958, 1964) to be a major causal factor, do not appear to be involved from the results of my study.

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COMPOSITION OF THE DISSOLVED ORGANIC COMPOUNDS IN SEAWATER: A REVIEW

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ABSTRACT

A review has been made of the chemical individuals and types of compounds that have been identified, isolated or reported present in seawater. Improvements in methods for determining the composition of the dissolved organic compounds may be important new tools for ecological investigations. There are few rapid, simple and accurate procedures suitable for large-scale surveying of seawaters. Evidence supporting Lucas' hypothesis of the role of dissolved organic compounds in seawaters may now be considered overwhelming.

INTRODUCTION

Dissolved and suspended organic matter are the major determinants of the biological difference between natural seawater and saline solutions of the same mineral composition. These differences can be demonstrated physically, chemically and biologically. Color and optical transparency of natural seawater were among the earliest indicators of a distinction in natural and artificial seawater. "Gelbstoffe" is sometimes used to name these unknown yellow substances (*humic substances* is another synonym equally meaningless) in seawater. Natural seawater has certain foaming properties not shared by the equivalent saline solutions. There are natural slicks on the sea surface in various places and at infrequent times which exhibit marked differences in surface tension as compared with

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either ordinary, natural seawater or artificial seawater. The organic constituents of natural seawater probably account for the peculiar solubility relationships of normally insoluble substances; *e.g.*, calcium carbonate, heavy metal hydroxides, oxides and sulfides, etc. Organic compounds may function as protective colloids for suspended particles of highly insoluble material, or coat soluble materials and thus forestall their solution. Some types of suspended organic matter can adsorb certain trace metallic ions from aqueous solutions. Such adsorption serves effectively to concentrate the ions and may conceivably supply necessary food needs to certain plants and animals.

These physical and chemical differences in seawater accentuate the biological differences. Few organisms will continue growth and proliferation in salt solutions identical in all respects with natural seawater, but free of organic constituents. Lucas (1936, 1938) has shown that the waste materials of one organism might be useful to its neighbor as a vitamin or hormone, or valuable to itself as a defense against predators or its competitors. The antibiotic activity of seawater towards some kinds of bacteria is fairly well-attested (Pramer, Carlucci and Scarpino 1962; Jones 1962; Steemann Nielsen 1961).

Possibly the most important aspect of study of the organic constituents of seawater lies in finding a new dimension to our understanding of ecology in the sea. This is accomplished by measuring the productivity (*i.e.*, the quantity of carbon dioxide entering the photosynthetic cycle per unit time), the transport and metabolism of photosynthetic products (*i.e.*, the composition of the organic matter that is actually consumed by plants and animals in living, reproducing and dying) and finally mineralization (*i.e.*, conversion of plants and animals and their waste materials into their inorganic constituents). The middle step, the transport of photosynthetic products and consumption by plants and animals, is very poorly understood. It contains the common threads of the oxygen, nitrogen, phosphorus and sulfur cycles in nature, but the specific chemical individuals that pass through the system are not very well known.

Bacteria consume and degrade the dead plants and animals, supply some nutriment to larger plants and animals by fixing nitrogen and complete similar ecological tasks. Each step in the food chain involves digestion or dissolution into comparatively simple organic compounds. These substances enter into the composition of seawater as transients, but exist as dynamic intermediates in the natural homeostasis of the sea. There is a widespread belief that these dissolved organic materials in the sea are uniquely refractory substances, though most of the identified compounds are recognized as being extremely mobile in living processes. Owing to the very low concentration of dissolved organic substances, theorists have sought to find physical means of concentration to account for this material being the trophic base for life in the sea. Dissolved organic substances have been demonstrated to be adsorbed by both organic and inorganic detritus, particulate matter is produced when seawater is aerated and concentration disconformities are found at the sea surface and at thermo- and halo-clines. It has been suggested that the dissolved organic matter in seawater serves solely as a supply of vitamin or hormonal growth factors rather than provide the direct energy requirements of particular organisms. An elegant demonstration of this kind of effect is afforded

by Pascher (1931). He placed a short piece of white thread to float in a dish containing female filaments with ripe eggs of *Sphaeroplea*. After floating in the dish for a time, the threads were transferred to another dish containing motile antherozoids. Soon thereafter, there was a marked accumulation of antherozoids around the threads. This persisted sometimes for an hour or more before disappearing. Idler, Fagerlund and Mayoh (1956) found that the migrating salmon can recognize extraordinarily low concentrations of L-serine, an amino acid found in human hand water rinses. More recently, Tester (1963) observed that the same amino acid repells sharks.

Interest in such investigation may be illustrated by the work of Wilson (1951) on "good" and "bad " seawaters. He had great difficulty in carrying cultures of various annelid seaworms through several generations in the seawater from around Plymouth, England. Two indicator species, *Sagitta setosa* and *Sagitta elegans*, were used to characterize the different waters. Water favorable to *S. elegans* was useful in supporting both plankton and the seaworms. Water that was favorable to *S. setosa*, on the other hand, caused the seaworms to develop abnormally. Introducing vitamin B_{12}, ascorbic acid, ethylenediaminetetraacetic acid (a chelating agent, abbreviated EDTA), antibiotics, etc., did little to improve the abnormal character of the water. Change of acidity, sterilization by ultrafiltration through special membrane filters, extraction of the water with activated carbon or solvents and other treatments appeared to have little influence on the biological properties of the water. The problem became one of identifying the nature of the organic constituents of seawater which distinguish a biologically "good" water from a "bad" water (Wilson 1951; Wilson and Armstrong 1952, 1954, 1958).

The naive analytical approach (*viz.*, separating the organic matter and subjecting it to a definitive chemical analysis) is beset with serious practical difficulties. The concentration of organic matter is very low, only 1–2 mg/l. The most efficient procedures for separation and isolation tend to be also the best procedures for formation of artefacts. Particulate matter is usually separated by membrane filtration, but the filters "bleed" carbohydrates of indeterminate nature into the water during use. A practical means is needed to concentrate the different dissolved organic constituents of seawater in sufficient quantities to permit conventional chemical microanalysis. There is little prospect at the present time that any single means will be found, owing chiefly to the diversity of chemical types involved; *e.g.*, carbohydrates, hydrocarbons, lipids, amino acids, various soluble polymers, organic nitrogen and phosphorus compounds, metal-ligand coordination complexes, etc. Large-scale desalination plants offer an opportunity for recovering a variety of products with fairly good reliability. Thus, plants where magnesium hydroxide is precipitated could be expected to provide the coprecipitated organic constituents in the crude magnesia. Electrodialysis plants, on the other hand, might be more useful in providing concentrates of soluble, high-molecular weight substances. The organic concentrates from 1–10 thousand gallons will be sufficiently large to allow detailed chemical and physical examination. Techniques of partition chromatography, gel filtration, gas chromatography, thin-layer chromatography, high-resolution mass spectrometry, nuclear magnetic

resonance spectroscopy and infrared spectrophotometry can separate and identify extremely small quantities of organic compounds with precision virtually beyond the dreams of investigators of only one or two decades ago. Thus far, these powerful new tools have not been systematically utilized in solving the problems of the composition of the dissolved organic substances in seawater.

An alternative approach is to examine the natural metabolic products of plankton grown under controlled conditions, then determine the course of these metabolites to their ultimate mineralization. Biological organisms are more sensitive to individual chemical species than are the more common chemical techniques, hence such an approach offers some very real advantages. New physical techniques (*e.g.*, isotopic tracing, electrophoresis, etc.) now allow more to be learned about the pathways of dissolved organic constituents than has been possible heretofore.

DEFINITION OF DISSOLVED ORGANIC CARBON

The first difficulty in analysis of dissolved organic substances lies in defining "dissolved organic carbon." There is no question that there is more dissolved and suspended matter than there is living matter. Plankton nets can be used to separate the larger organisms and the smaller can be removed by simple filtration or centrifugation. Very fine filters are required to remove the smallest of the plankton and at this point the distinction between dissolved and colloidal substances becomes important. Substances with enzymatic activity may or may not be living and they may or may not be associated with formed bodies. The suspended particles can be classified into 7 major groups; *viz.*, 1) algae, 2) bacteria, 3) organic colloids including viruses, 4) organic macromolecules, 5) suspended inorganic particles, 6) colloidal inorganic particles and 7) dissolved inorganic particles. Lammers (1965) managed to separate some of these groups by continuous or density-gradient centrifugation. It is possible to distinguish density differences of the order of 0.005 g/cc with these methods. Generally, the ratio between the detritus and living organisms is assumed to be approximately 300:1 (Krogh 1931, 1934) to 500:1 (Parsons and Strickland 1962). An absolute value cannot be obtained because many of the smaller organisms attach themselves to the nonliving particles and escape segregation. About 70% is "crude fiber" carbohydrate, insoluble in either acid or alkali. There is not much chitin or hexuronides from marine plants, contrary to expectation.

Most investigators now apply as a rule-of-thumb the doctrine that passage through a 0.45 μ "Millipore" filter (trade-mark of Millipore Filter Corp., Bedford, Massachusetts) separates suspended matter from dissolved matter. Some investigators employ the 0.22 μ filter. Others use glass filters, activated carbon adsorption, centrifugation or gravitational settling. No definitive answer has been given to the question of the difference in the suspended matter and dissolved organic matter beyond the operational definition of experimental conditions employed in each particular circumstance.

There are several difficulties inherent in using carbohydrate filters. Guillard and Wangersky (1958) found the HA Millipore filters they used contained a

substance soluble in water that would give a positive test with N-ethylcarbazole. This is a test for putative carbohydrates. At least one liter of water was needed to remove all the elutable "carbohydrate." Cahn (1967) identified detergents in these membrane filters. Parker (1967) reviewed the problems and showed the differences in dissolved organic carbon produced by filtration through a 0.45 μ membrane, washed and unwashed, were significant. No significant differences occurred between 0.45 μ washed Millipore and 0.45 μ Selas silver membranes, though the latter had lower throughput. Considerably less organic matter passed through unwashed 0.22 μ Millipore membranes than through unwashed 0.45 μ membranes, as might be anticipated. It is not known whether the effect is due to adsorption or filtration.

The heterogeneity of particulate matter in natural waters is nearly incredible. Lammers (1965) observed particles ranging in size even below 10 mμ up to 0.5 μ, having sedimentation rates of 1 to 10^5 Svedberg units. There is little wonder that chemists have, as a rule, followed the simple, operational criteria for designating detritus. Menzel and Vaccaro (1964), for example, recommended filtration with pre-combusted glass filters, rinsed with a small quantity of the sample before use.

There are also problems connected with determination of the total dissolved organic carbon. Early investigators would precipitate chloride ion with silver, then oxidize the organic constituents in the filtrate with either alkaline permanganate or chromic acid. Raben (1910) filtered the freshly collected seawater through a Berckefeld filter and carried out the combustion analysis within two days. He used a 100 ml sample, and determined the CO_2 by weighing barium carbonate. Krogh and Keys (1934) filtered the seawater through membrane filters in the Thiessen Ultrafiltration Apparatus to remove detritus and colloids. The halides were separated by precipitation with thallous sulfate, the seawater residue evaporated to dryness and oxidized with ceric-chromic-sulfuric acid. The CO_2 evolved was taken up in barium hydroxide and weighed. Kay (1954) oxidized filtered seawater with a silver-containing mixture of chromic and sulfuric acid. The oxidation product was scrubbed with potassium iodide to remove any chlorine that was accidently passed over, then the vapors conducted over a platinum gauze to oxidize any residual carbon monoxide to CO_2. Gillbricht (1957) has improved upon the alkaline permanganate methods, but Szekielda (1968) has found permanganate consumption to bear no relationship to the quantity of dissolved organic carbon in the water.

Armstrong, Williams and Strickland (1966) introduced the photoöxidation method to destroy all the organic constituents of seawater. They used several hours of exposure to a powerful ultraviolet radiation source to effect photoöxidation of the dissolved organic compounds. Recently, Armstrong and Tibbits (1968) extended this procedure to include determination of nitrogen, phosphorus and carbon. Though time-consuming, this procedure is probably one of the most reliable (*i.e.*, high precision and accuracy) known.

Wilson (1961) introduced persulfate oxidation as a fast means for converting all of the organic carbon into CO_2. The method was refined by Menzel and Vaccaro (1964) into a comparatively simple, speedy procedure:

Inject 5 ml of seawater through a 2.4 cm glass-fiber filter into a 10 ml pyrex ampoule (pre-combusted in a muffle furnace at 700 C). The filter must be rinsed with 5 ml of sample before use. Add 0.1 g potassium persulfate and 0.1 ml 3% H_3PO_4 into the ampoule. Bubble nitrogen through a cannula into the ampoule at 200 ml/minute for 3 minutes to remove the inorganic carbon. Place a plug of silicone grease in the end of the ampoule and seal in an oxygen-gas flame. Autoclave the samples at 130 C for 30 minutes. After cooling, the neck of the sealed ampoule is inserted into a rubber tube and nitrogen flushed through the system to remove extraneous CO_2. Shut off the gas flow, crush the ampoule tip and insert the cannula into the solution. The CO_2 may be measured either by infrared absorption or any of several other means.

Williams (1966) tested the completeness of the oxidation—a handicap of the chromic oxide and potassium permanganate oxidation methods of the past—by adding a known quantity of radioactive glucose or amino acids to the sample. The residual radioactivity in the solution was determined after the oxidation was completed. Large variations in concentration of the organic material had little influence on the residual radioactivity. Amino acids are less fully oxidized by the reagent than glucose. The residual radioactivity for proline is 22.0%, arginine is 4.4% and leucine is 6.5%. No particular effect was noted for the catalytic action of copper, use of nitrogen instead of oxygen to purge out the CO_2 or increase of either persulfuric or phosphoric acid concentration. The temperature of oxidation is important. At 80 C, 6.9% of the added radioactive carbon remained, while at 100 C, only 1.8% remained. At still higher temperatures (*e.g.*, 130 C), 3.0% of the radioactivity remained with the residue. The most extensive oxidation occurred at 100 C instead of at the higher temperatures. Kinetic curves showed little further breakdown of amino acids after 2.5 hours at 100 C.

There is at present no evidence of the reason for the incomplete oxidation of amino acids, as compared with fatty acids or carbohydrates. The limited quantity of persulfuric acid may decompose thermally before it can contact all of the organic substrate to be oxidized. Addition of still greater amounts of reagent would increase the blank. The amine group can possibly be oxidized at room temperature with persulfate to the amine oxide. At a higher temperature, the amine oxide may possibly decompose to furnish the corresponding olefin, which is subject to polymerization in the presence of decomposing persulfate. Thus, alanine might give acrylic acid and thence a polymer of acrylic acid that would be far more difficult to oxidize than its precursors. These speculations are unsupported and need verification.

A procedure with great potential value, should it be refined to higher sensitivity, has been worked out by Van Hall, Safranko and Stenger (1963). They inject the water sample into a combustion tube where the organic matter is oxidized to CO_2 in a current of oxygen. The gas stream is passed through an infrared analyzer designed to measure CO_2 and the signal recorded. The large volume of steam produced limits the sample size, which itself limits the concentration level of dissolved organic carbon to about 2 mg. Samples of about 20 μl are generally used. Larger samples present risk of excessive internal pressures.

Smaller samples are limited by the lower reproducibility of the volume measurements. Although this method has the advantage of speedy operation, it does not deal successfully with the low concentration levels found in seawater. Modifications may be possible so as to permit its application to the low concentration level found in seawaters.

Lysyj and Nelson (1968) combined pyrolysis with gas chromatography as a means of determining the organic content of solutions in the parts per million range. The sample is pyrolyzed in the presence of the matrix water and the pyrolytic fragments transported by steam as the carrier through a glass bead column. No separation is achieved and the total pyrolysate is measured as a single peak on the hydrogen flame ionization detector. Although this procedure appears promising, there are problems of standardization and control of the equipment, and it must be verified on seawater samples that have been analyzed by other methods. This method does not appear to have been employed in the analysis of seawaters as yet.

Soviet investigators have favored dry combustion methods. Krylova (1957) pyrolyzed the organic matter of seawater samples in a deficiency of oxygen, then catalytically oxidized the gases in the presence of silver and platinum. This procedure, however, failed when applied to samples from the ocean because of magnesium chloride. Skopintsev and Timofeyeva (1962) worked out a variation that appears to be fairly fast and reproducible:

> Decompose bicarbonates by adding 0.1 *N* H_2SO_4. Determine the quantity needed by titrating a separate sample with mixed Methyl Red-Methyl Blue indicator, in a flask fitted for alkalimetry in a current of CO_2-free air. Evaporate the seawater samples at 50–60 C in glass evaporating dishes on a steam table. Place the evaporating dishes containing the dry residue in a desiccator over concentrated H_2SO_4 before analysis. Handle all equipment and vessels with tongs, platinum hooks, on clean surfaces to avoid contamination. Pyrolysis and combustion is done at 700 C for 10 minutes in a current of CO_2-free air at 5 ml/min. Bring the furnace up to 900 C. Liquid hydrochloric acid, formed in the pyrolysis and combustion, flows towards the narrow end of the tube. Expell the CO_2 with the same air flow over a period of 10 minutes following. The air rate is increased and the excess $Ba(OH)_2$ in each of the 3 absorbers is titrated with standard 0.02 *N* HCl. (After titration, the barium hydroxide absorbers may be used over again.)

This procedure is apparently not used except by Soviet investigators. Their values for the organic carbon dissolved in the waters of the Baltic, North Sea and various regions of the North Atlantic and Mediterranean Sea differ from those obtained by other workers. They verified their procedure by adding known quantities of sucrose and phenylalanine to seawater samples and measuring the known addition of carbon. The procedure has yet to be compared systematically with other methods; *e.g.*, photoöxidation or persulfate oxidation. Carbon contents of various seawater specimens collected in the Northeastern Atlantic gave values of 1.05 to 1.96 mg/l. Little variation in dissolved organic carbon was found with depth (Skopintsev 1963). Most other investigators have found the dissolved

organic content varied with the depth, with higher values near the surface. Few have reported concentrations from the open, putatively unpolluted sea of greater than 1.2 mg/l. Duursma (1960) determined dissolved organic carbon in various depths and showed that layers exist where there are comparatively higher quantities of carbon both above and below, confirming the earlier studies by Plunkett and Rakestraw (1955) that variations exist. In the course of a year, Duursma (1963) observed marked variations in the rate of formation and decomposition of organic matter in the sea.

GENERAL METHODS FOR ISOLATION OF ORGANICS

Problems common to all isolation procedures are the biological mobility of the soluble organic substances, their thermolability, the overwhelming mass of inorganic dissolved substances and the ready, irreversible adsorption of dissolved organic substances at surfaces. Jeffrey and Hood (1958) compared five different methods of isolation or recovery of organics from seawater; *viz.*, dialysis, adsorption, ion exchange, solvent extraction and coprecipitation. They employed a C^{14}-labelled aged algae culture as the test solution to evaluate the various isolation and separation methods.

Ordinary dialysis can be used to remove the salt from seawater. A cellulose acetate membrane is placed between distilled water (or merely nonsaline water at the outset) and the seawater. The inorganic ions migrate into the lower concentration through the membrane, while the organic substances have a far less tendency to pass through the membrane. Some small organic molecules will pass through, however, and some of the ions do not pass through in proportion to their concentration. By stopping dialysis before complete desalting, essentially all the organic substances can be retained. The concentrate may then be flash evaporated under reduced pressure and dialyzed again. Certain compounds (*e.g.*, ascorbic acid, alanine and phenylalanine) are poorly retained in dialysis, though C^{14}-labelled compounds were completely recovered. New membranes bleed organic carbon into the water, hence all membranes must be presoaked and washed before use.

Electrodialysis appears to give essentially the same results as ordinary dialysis but with greater speed. When used with ion exchanging films as the membranes, electrodialysis can reduce salinity very rapidly. The concentrates obtained after flash evaporation were yellowish-brown solids, partly soluble in distilled water. The substances were insoluble in chloroform, ether, petroleum ether, CS_2, CCl_4 and acetone, but soluble in dilute alkali, methanol, dimethyl sulfoxide, etc. Electrochromatography of the concentrate, buffered to pH 7.0, showed positive, negative and neutral fractions. Spray tests for sugars and amino acids were negative and the ultraviolet and infrared spectra were poorly characterized.

The chief disadvantage of dialysis methods lies in the large volumes of water which must be removed. Low temperature evaporation or freeze drying may be used. It may be possible to obtain organic concentrates from large-scale seawater desalting plants by low-temperature evaporation of the water.

Adsorption on carbon is an efficient way of removing organic matter. No fully

satisfactory method has thus far been devised to desorb the organic matter completely again. Part is recovered by refluxing with phenol, but some impurities from the carbon are removed at the same time. Gradient elution with aqueous ethanol solutions allows separation of various low molecular weight carbohydrates, but amino acids are lost or destroyed unless the columns are pretreated with HCN and acetic acid.

A fascinating type of chromatographic column made from nylon stockings has been described by Sieburth and Jensen (1968) as being particularly valuable for concentrating "Gelbstoff" from seawater. These columns are made by folding the tightly-rolled, wet stocking into a 100 ml open separatory funnel. Such columns consistently adsorb 70% or more of the humic pigments, practically all of which could be eluted with 0.1 *N* NaOH solution. Polyamide powders [*e.g.*, Divergan SZ 9010 and Ultramid (Badische Anilin und Sodafabrik), MN-Polyamides 2117 (Machery, Nagel und Cie) and Polyamide powder (Farbwerke Hoechst)] were all found to bind as much as 30% of the yellow pigments irreversibly.

Paper chromatograms of the separated "Gelbstoffe" demonstrated its composite nature and the differences between "Gelbstoffe" from bogs and rivers and that from the sea. The microbial dextran, "Sephadex", was employed by Gjessing (1965) to separate and characterize the high-molecular weight humic substances from natural waters. Some of these substances appear to be ferrous and calcium complexes. Khailov (1968) fractionated the organic polymers present in seawater by gel filtration through "Sephadexes". He isolated 9.2 mg from about 20 liters of seawater.

Seawater may be demineralized with a combination of ion exchange resins. Large quantities of resins are needed to separate comparatively small amounts of dissolved organic matter. The resins must be soaked and washed very carefully to avoid organic contamination from the resins. Organic acids are taken up and certain carbohydrates may be transformed by contact with the more strongly basic resins. The volume of liquid recovered is often twice as great as the seawater sample taken. Jeffrey and Hood (1958) suggest that ion exchange resins will probably have their greatest merit in the final separations of partially desalted organic concentrates obtained by other methods. A strong cation and a weak anion exchange resin appear to be better for isolating the aged C^{14}-marked algal materials than any other combination of resins.

Carritt (1953) suggested a sort of partition chromatographic column made of cellulose acetate loaded with dithizone dissolved in carbon teterachloride. The heavy metals would tend to concentrate in the dithizone loaded column and possibly the metals might retain their organic chelants in the course of separation. Similar ideas have been repeated more recently (Carritt 1965).

Jeffrey and Hood (1958) studied liquid solvent extractants (*e.g.*, phenol, ethyl acetate and 2, 6-lutidine) for concentrating organic substances from seawater. Owing to the low concentration of the dissolved organic matter, extraction requires many stages to realize very limited quantities of dissolved substance in recovery. The solubility characteristics of the suites of dissolved organic substances are so diverse that any single solvent can, in principle, recover only a small fraction of the total under the best of circumstances. Without prolonged

extraction in many stages, it is unlikely that quantitative recovery can be accomplished. If one accepts the limitation of partial recovery, solvent extraction offers the advantages of fast recovery with comparatively little destruction of the molecular species present. Speed, however, is not characteristic of the recovery of organic acids. Koyama and Thompson (1964a, 1964b) showed that continuous extraction with chloroform for one week gave only 80% recovery of acetic, formic, lactic and glycolic acids and that 95–100% recovery was obtained after 3 weeks of continuous extraction. Not more than 5% of the malic and citric acids added, however, were recovered even after 3 weeks. Ether gave systematically lower recoveries. Carbon disulfide was used by Williams (1961). Khailov (1962) used phenol to extract complex compounds from seawater. Goncharova and Stradomskaya (1965) have reviewed the various methods employed for separate determination of acids in natural waters.

By raising the pH of seawater to 10, a substantial quantity of the dissolved organic matter is coprecipitated with magnesium hydroxide and calcium carbonate. Even higher yields of dissolved organic matter can be recovered by adding ferric chloride and precipitating ferric hydroxide at pH 7.6–8.2 (Tatsumoto *et al.* 1961; Park *et al.* 1962; Williams 1961). As little as 0.1 mg/l of dissolved organic matter in deep-sea water was recovered by Williams by co-precipitation with 20 mg/l ferric ion. Good recoveries were obtained when known organic compounds were added. Inorganic clays adsorb starch and other carbohydrates (Lynch, Wright and Cotnoir 1956; Bader, Hood and Smith 1960), peptides and amino acids (Sieskind and Wey 1959). Williams and Zirino (1964) measured the efficiency of recovery by determining the dissolved organic carbon before and after coprecipitation. In a very careful investigation, they showed ferric hydroxide more efficiently scavenged organics than any other system examined; *e.g.*, adsorption by filter pads of activated alumina, celite-magnesium oxide or silicic acid. Under optimum conditions, 20 mg/l ferric ion, pH 4–6 and 4–8 hours settling time, about 60–63% of the dissolved organic carbon was removed. A five-fold increase in ferric ion afforded only 12% increase in carbon recovery. Water from the sea surface was more completely freed of dissolved organic matter by this treatment than deep-sea water. The deep-sea water presumably had already been exposed to natural adsorptive and coprecipitation processes and its dissolved organic matter thus were more refractory under these conditions. Jeffrey and Hood (1958) had used aged marine algal cultures which had been incubated with radioactive CO_2 in their work, hence it is possible that the dissolved organic matter coprecipitated in their studies differs from the dissolved organic matter normally present in seawaters. Chapman and Rae (1967) collected some seawater, filtered it immediately through 0.45 μ Millipore filter and the filtrate sterilized by adding mercuric chloride. Later, 2.5 mg/l ferric chloride was added and precipitated with 2 *N* sodium hydroxide, the precipitate taken up in HCl and chromatographed on an ion exchange column. A broad range of organic substances were added (*e.g.*, glucose, sucrose, lysine, leucine, proline, glycine and glutamic, citric, lactic, malic and glycollic acids, starch, gum acacia and blood albumin) and their recovery attempted. Only glutamic acid was recovered in good yield. Each amino acid appears to have an optimum pH for its coprecipitation with ferric ion. Copre-

cipitation with copper at pH 9 gave a marked improvement at amino acid recovery. The addition of borate did not improve coprecipitation of glucose, but glycollate recovery was increased to 37% at pH 3.5–6.0. Albumin and carboxymethyl cellulose synergize coprecipitation of glycollate but do not affect glycine, lysine or glucose. Akiyama and Hanya (1968) were able to recover only about 30–50% of the dissolved organic matter in natural waters by coprecipitation of ferric hydroxide. They confirmed spectroscopically that the product resembles the bacterial degradation product of the green alga, *Scenedesmus*.

The organic substances, particularly organic acids, can be separated from the precipitates by dissolving in hydrochloric acid at pH 2, passing the solution through cation exchange resin and eluting with 0.1 *N* HCl. The pH is adjusted to 3.5 and the solution evaporated to dryness *in vacuo*. The residue is extracted repeatedly with isopropyl alcohol or similar organic solvent. The crude extracts are light yellow in color and have a blue-green fluorescence (Williams 1961).

Bader, Hood and Smith (1960) studied adsorption of C^{14}-tagged organic compounds; *e.g.*, sucrose, glucose, fructose, alanine, and acetic, benzoic, oxalic, succinic, butyric, aspartic and glutamic acids, valine and glycine on kaolinite, montmorillonite and illite. These suspended sedimentary particles adsorbed comparatively little of the dissolved organics. The mineral particles had a characteristic effect on the solution, nevertheless. Button (1969) confirmed that montmorillonite, particle sizes from 1.5 to 2.5 μ, does not render the organic matter unavailable to microorganisms or remove it significantly from solution. Neither glucose nor thiamine is taken up by the clays. It is interesting to note that radioactive ionic calcium is less dialyzable in seawater containing organic matter than in seawater free of organics. Apparently the calcium is bound in some manner to the organic material. This is a complicated problem because clays appear to scavenge phosphates, calcium, iron and many other plant nutrients.

Chave (1965) observed that there is essentially no interaction between calcium carbonate and seawater, probably due to the presence of resistant organic coatings on the particles. Chave and Schmalz (1966) suggest that abrasion in surf or other zones of high wave action may be important in promoting simple chemical interactions that are usually forestalled by the organic coatings. The protein matrix appears to be a determining factor in deposition of either aragonite or calcite in the shells of molluscs (Watabe and Wilbur 1960; Wilbur and Watabe 1967). This matrix, conchiolin, was hydrolyzed by Tanaka, Hatano and Itasaka (1960) and its amino acids estimated chromatographically. Relatively large amounts of leucine, alanine, glycine and cystine were found. This composition differs but little from the amino acids of coccoliths (Isenberg *et al.* 1965), which were also determined chromatographically. Hare (1963) demonstrated that systematic variations occur in the amino acid composition of the other prismatic layer and that the calcitic layers have a consistently higher ratio of acidic to basic amino acids than the aragonitic shell units. He speculates that "Certain side groups in the protein matrix may concentrate Ca^{++} and $CO_3^{=}$ in specific positions and thus provide an appropriate initial concentration of these ions to nucleate the mineral phase. Aspartic and glutamic acid side chains could provide negatively charged sites, which would attract calcium ions. Similarly, the basic side chains could

provide sites for the concentration of carbonate (or bicarbonate) ions. The fact that the nonmineralized units have very few acidic residues may indicate a possible mechanism for preventing mineralization." Kobayashi (1964) sampled some of the extrapallial fluid from 14 different species of molluscs and examined the protein characteristics by paper electrophoresis. Species with exclusively calcitic shell have an extrapallial fluid with but a single protein fraction. Those with an aragonite shell or shells with both crystalline forms in them have three or more different protein fractions. Acid mucopolysaccharides and possibly neutral heteropolysaccharides are also found in the extrapallial fluid. Some species contain sulfated mucopolysaccharides.

Siegel and Degens (1966) called attention to the possibility of concentrating and removing amino acids from solution by ligand exchange chromatography. Cation exchange resin saturated with a complex-forming heavy metal (*e.g.*, copper or zinc) acts as a solid sorbent. Ligands may be anions or neutral molecules. The latter attach themselves to the resin through coordination with the metal on the resin. By this technique, concentration of organic ligands is possible from very dilute solutions, even in the presence of competing inorganic ligands. Seawater is such a very dilute system. Siegel (1967) has discussed the theory of ligand exchange chromatography and illustrated its application by using Chelex-100 with zinc. This technique is particularly effective in concentrating amino acids, peptides, purines and pyrimidines. There is a good chance it will serve successfully for determining algal excretion products, humic acids and phenols. Ferric columns have been shown to pick up 70% of the organic phosphorus compounds from seawater, along with nearly all of the inorganic phosphorus compounds. 2-Aminoethylphosphonic acid, a compound containing a carbon-phosphorus bond, has been isolated from numerous marine animals (Quin and Shelburne 1969) and might be possibly identified or separated by ligand exchange chromatography.

Shapiro (1961) proposed freezing as a method for quantitative recovery of small concentrations of organic constituents in natural waters. The technique is attractive because good recoveries can be obtained of both volatile and heat-labile substances. He asserted that recoveries greater than 99% were obtained. Kobayashi and Lee (1964) examined freeze concentration of dilute solutions of Rhodamine B in sodium chloride solutions. The dye recoveries ranged from 82.3% to 94.2%. The operation of freeze concentration appears to be complicated by entrapment of particles as a result of super-cooling. They suggest that recovery efficiency depends on the nature of the solute rather than its concentration. Baker (1967a, 1967b) has worked out a freezing procedure for separation and concentration of organic substances from fresh waters. The presence of inorganic salts impairs recovery efficiency, hence its use for seawater depends upon a preliminary demineralization. Freezing concentration is potentially a valuable technique, particularly for volatile or labile substances which may be lost, chemically altered or selectively separated by conventional methods, but it will require further study and development before it becomes practical for seawater.

Collier *et al.* (1950, 1953) showed that certain organic substances, probably carbohydrates, caused oysters to initiate or cease pumping. Because of the ex-

tremely low concentration of the dissolved organic compounds, it has been considered very improbable that any organisms would actually employ the dissolved organics for food. Stephens and Schinske (1961) proved that several amino acids are taken up from dilute solutions by a variety of marine invertebrates. The solutions, however, were considerably more concentrated than the dissolved organic matter in ordinary seawater. Brackish water worms (*e.g.*, the annelids *Nereis limnicola* and *N. succinea*) take up glycine (Stephens 1964). Chapman and Taylor (1968) demonstrated that *Nereis virens* from the Thames Estuary takes up glutamic acid from the water. The deep-sea animals of the new phylum *Pogonophora*, dredged from 100 fathoms off Miami Beach, Florida, appear to consume amino acids from the seawater (Little and Gupta 1968). *Siboglinum mergophorum* took up radioactive phenylalanine at concentrations from 2×10^{-6} to 2×10^{-8} *M*. Blood samples taken from the animal *Oligobrachia floridana* actually contained some of the tagged phenylalanine shortly after taking it up from the seawater media. Macromolecular species were also taken up by this phylum. Stephens (1960, 1962, 1964), also using C^{14} tracer, found the solitary coral, *Fungia*, was capable of removing several small organic molecules of biological significance from very dilute solutions. For this particular organism, the glucose removed is significant when compared with the energy requirements of the animal. Ambient concentrations of approximately 15 mg/l would provide sufficient organic matter to support the observed oxygen consumption.

Microbiological methods, when suitably validated, are extraordinarily effective in determining minute quantities of biologically significant metabolites in seawater. Provasoli and Pintner (1953) assayed vitamin B_{12} with the flagellate, *Euglena gracilis*. Seawater must be diluted to reduce the salinity and permit *Euglena* to grow satisfactorily. The chlorophyll is extracted and determined colorimetrically (Daisley 1958). The red alga, *Goniotrichum elegans*, requires the B_{12} vitamins and cannot utilize cobalt as a replacement (Fries 1959). Burkholder and Burkholder (1956) estimated the B_{12} vitamins in various suspended marine solids by a microbiological method. Biotin and thiamine can be determined with the alga, *Gyrodinium cohnii* (Provasoli and Gold 1959). A wide variety of organisms can be used to determine B_{12} (Carlucci and Silbernagel 1966, 1969). Faster and more convenient than earlier assay organisms, *Serratia marinorubra* was introduced by Carlucci and Belser (1963). Belser (1958, 1959) found this organism can be mutated with ultraviolet radiation to give strains that have many unique biochemical deficiencies. Litchfield and Hood (1965, 1966) developed its use for determination of biotin distributions in the Gulf of Mexico and the assay of adenine, uracil and threonine. Carlucci and Silbernagel (1969) have prepared a list of marine phytoplankters that require B_{12}, thiamine and biotin for laboratory culture.

Bentley (1958, 1959) noticed that extracts of marine phytoplankton had an effect on oat coleoptile lengthening; *i.e.*, some fractions of the extracts have plant hormone activity. "This suggests that some organic compound or compounds other than the major nutrients necessary for growth is required by the organisms and leads to the idea that the distribution of these organisms in their native environment may be controlled by these trace substances, at least in part."

Mineralization of organic compounds (*i.e.*, conversion of the substances into water and carbon dioxide) has been studied by collecting the particulate matter (seston) and subjecting it to combustion analysis. Since the quantity of dissolved organic substances is obviously greater than the quantity of seston, methods have been sought for a more direct study of mineralization. Attempts have been made to estimate the oxygen consumption, but without great success in elucidating the extent of mineralization because of the difficulty in separating respiration from photosynthesis in a living system. Wright and Hobbie (1965a, 1965b) measured the rate of uptake of C^{14}-labelled organic compounds by plankton. Bacteria evidently take up the organic substrate by a process different from that used by algae. The specific transport systems used by bacteria seem to be dependent upon the nature of the substrate and were operable at concentrations below the diffusional process employed by algae (Wright and Hobbie 1966). Vaccaro and Jannasch (1967) ascertained the applicability of uptake kinetics for assessing the heterotrophic activity of microorganisms. They measured the passage of glucose labelled with radioactive carbon from the solution into the organisms. Since this technique may have value in determining the efficiency and turnover time for organic substrates as well as assimilation, it was studied with both Atlantic and Pacific seawaters (Vaccaro and Jannasch 1968). Williams and Askew (1968) worked out a direct method for estimating conversion of organic matter into CO_2 using C^{14}-tagged substrates. Small amounts of the uniformly labelled material is added to the seawater and incubated for 3 hours. The sample is acidified with phosphoric acid, degassed with CO_2-free air and the CO_2 recovered as barium carbonate. The CO_2 generated from the substrate is a measure of the respiration during the 3-hour incubation period. Though glucose was used in this work, it is evident that any suitably labelled organic compound may be studied. They suggest, "If it were possible to prepare a mixture of radioactive compounds of the same composition as the organic compounds present in sea-water, then the total amount of microbial respiration in a sample of water could be measured. This is, of course, not possible at the present and will probably never be so. Nevertheless, the field of marine organic chemistry is attracting considerable effort currently and it is probable that we may soon be able to predict the approximate amino acid, fatty acid and sugar composition of a given body of sea-water. This would permit the preparation of mixtures of the principal amino acids, sugars and fatty acids labelled with ^{14}C. If small amounts of these mixtures were added to replicate samples of sea-water, the mean turnover time of each of the above groups could be determined as described. . . ." Seki (1964, 1967a, 1967b, 1968) has developed a microbiological method for the study of mineralization rates. A 100–1000 ml sample is filtered through Millipore Filter HA quickly, the collected material crushed into a 30 ml sterile glass bottle containing 10 ml of sterile seawater and 0.4% peptone. The bottle is sealed and CO_2 measured by a modified Van Slyke respirometer, over a period of up to 6 hours. The CO_2 liberated is assumed to be the mineralization rate. This method has been used for examination of the ecology of organic matter in the Aburatsubo Inlet, Japan. The addition of small amounts of glucose or peptone has a strong effect on the CO_2 uptake in the dark. Szekielda

(1967, 1968) worked out a chemical model for buildup and degradation of organics with the stoichiometry in the sea:

$$(CH_2O)_{224}(NH_3)_{29}(H_3PO_4) + 282\ O_2 = 224\ CO_2 + 253\ H_2O + 29\ HNO_3 + H_3PO_4 \quad (1)$$

It is clear that study of the organic composition by isolation from the seawater is in its infancy. Though a number of specific compounds have been separated and identified from seawater samples, methodology remains the determinant of progress. Procedures have yet to be developed that are sufficiently simple, reliable, fast and accurate enough to permit widespread, systematic surveying of the organic composition of seawaters.

Alternative approaches (*e.g.*, the application of biological methods, measurement of kinetics of mineralization, etc.) have certain peculiar advantages and may have unique value for determining individual parameters in an ecosystem. Few of these methods have been sufficiently developed to be applied broadly to surveying the organic matter of the oceans.

Carbohydrates in Seawater

Carbohydrates have been characterized by reactions with anthrone and N-ethylcarbazole (Collier *et al.* 1953; Lewis and Rakestraw 1955; Collier 1953). Certain substances which are not usually classified as carbohydrates give positive tests with these reagents. Handa (1966) has critically examined the use of the phenol sulfuric acid test for determination of dissolved carbohydrates. Wangersky (1952) isolated a rhamnoside and crystalline dehydroascorbic acid from the waters of the Gulf of Mexico by adsorption on activated charcoal followed by elution with ethanol. Starch occurs widely distributed in the ocean as suspended particles (Bursa 1968), but it has not yet been demonstrated in colloidal solution. The starch particles are small grains from 0.5 to 10 μ with the largest grains having proximate diameters of 23–36 μ. Bursa suggests that dissolved starch derivatives alter the osmotic properties of water and influence the life processes of both zoo- and phytoplankton. Handa and Tominaga (1969) prepared a comprehensive analysis of the carbohydrates present in marine detritus. It is apparent that these carbohydrates are produced by the phytoplankton. Lewin (1956) grew several types of planktonic organisms in bacteria-free cultures and recovered the extracellular polysaccharides. After hydrolysis, the constituent sugars were fairly characteristic of the organism from which they were derived. For example: *Skeletonema costatum* appeared to produce no extracellular polysaccharide; *Nitzschia ovalis* gave off 5% polysaccharide containing galactose, rhamnose, xylose and uronic acid; *Chlamydomonas euryale* gave off 16% polysaccharide containing arabinose, galactose and minor amounts of rhamnoce and xylose; and *Porphyridium cruentum* yielded 50% polysaccharide containing glucose and xylose with lesser amounts of rhamnose, uronic acids and miscellaneous unidentifieds. It is perhaps significant that cellulose can be metabolized rapidly by various marine *Ascomycetes*. *Lulworthia floridana* and *Torpedospora* sp. can consume up

to 50% of a given specimen of cellulose in 3 weeks growth under laboratory conditions (Meyers and Scott 1968). Yeasts which metabolize carbohydrates have a wide distribution in the ocean (Fell 1967).

Degens, Reuter and Shaw (1964) found glucose, mannose and galactose in the seawaters off California. The anthrone method was used by Kawahara, Maita and Ishii (1967) for determination of carbohydrate in the waters of Southern Hokkaido. Hicks and Carey (1968) developed a sensitive, specific enzymatic assay for glucose suitable for application to seawater in concentration levels of the order of 10^{-8} *M*. Hexokinase reacts quantitatively with glucose to give glucose-6-phosphate, which is then acted upon by the enzyme glucose-6-phosphate dehydrogenase to furnish the reduced form of nicotinamide-adenine-dinucleotide phosphate (NADPH). The NADPH reduces the dye, resazurin, to a highly fluorescent product, resarufin. The quantity of resarufin is proportional to glucose in the original sample. The resarufin quantity is estimated with a fluorometer. Vaccaro *et al.* (1968) utilized this procedure for estimation of the glucose content of various Atlantic seawaters. They found that densities of phytoplankton were closely correlated with the glucose concentrations. Bursa (1968) suggests that starch in the oceans is a marker of past blooms or mass mortalities of phytoplankton. Changes of salinity may account for some starch liberation from phytoflagellates. Biggs and Wetzel (1968) have observed that carbohydrates tend to concentrate at the halocline in Chesapeake Bay. Concentrations of glucose by the enzymatic method have been confirmed by the independent bioassay procedure using an organism similar to *Achromobacter aquamarinus* (Vaccaro and Jannasch 1966).

The mechanism of carbohydrate formation in the sea is not known. Hellebust (1965) has shown that some 22 species of marine algae have some carbohydrate excretion during exponential growth and under high light flux. Most of them excreted 3–6% of their photosynthate. Glycolic acid formed from 9 to 38% of the carbon excretion in several of the species examined. Small amounts of polymers and chloroform-soluble molecular types were also obtained. Some species excreted almost exclusively, but a single molecular individual; *e.g.*, *Olistodiscus* made mannitol and *Dunaliella tertiolecta* gave chiefly glycerol. It is not entirely clear whether these excretion products are being produced by normal cell metabolism or by lysis of dead or dying cells. After the exponential growth phase levels out and the cells begin to age and deteriorate, there is no question that large quantities of dissolved organic matter appear in the water.

Carbohydrates, presumably monosaccharides, were characterized by the N-ethylcarbazole reaction among the excretion products of such marine flagellates as *Isochrysis galbana, Monochrysis lutheri, Prymnesium parvum, Dunaliella euchlora,* etc. (Guillard and Wangersky 1958). They observed the maximum rate of production after the exponential growth phase was completed. Under normal growth, comparatively little carbohydrate is excreted.

The extracellular products of algae were reviewed by Fogg (1962). Although most free-living algae can excrete soluble organic substances, symbiotic algae from corals, clams, anemones, etc., when grown in laboratory culture, excrete as much as 85% of their photosynthate as soluble carbohydrates (Muscatine 1965;

Muscatine and Hand 1958). McLaughlin and Zahl (1959) showed how to cultivate bacteria-free zooxanthellae from a variety of such host organisms. It appears that these algae are actually a phase in the life-cycle of otherwise free-living organisms. A detailed review of this kind of symbiosis is given by McLaughlin and Zahl (1966) and the pattern of carbohydrate transport is discussed comprehensively in a review by Smith, Muscatine and Lewis (1969). Muscatine (1967) crushed and ground fresh corals, strained the mixture and isolated the zooxanthellae by centrifugation. When grown in media containing C^{14}-labelled sodium carbonate, the carbohydrate given off contained an appreciable amount of the radioactive tracer. Up to 40% of the algal photosynthetic product was demonstrated to be glycerol from the reef coral, *Pocillopora damicornis*, and from the giant clam, *Tridacna crocea*. There appears to be no direct evidence that the external seawater is used as the media for exchange of the carbohydrate between these symbionts, but Goreau (1964) reported mass expulsion of zooxanthellae from Jamaican reef communities as a result of severe flood rains that accompanied a hurricane. Presumably, the loss of zooxanthellae occurred because of the lowered salinity and it is plausible to suspect that the expelled zooxanthellae would continue to live as they are known to grow in laboratory cultures. Such free-living algae would liberate polyols and other carbohydrates into the pool of organic substances normally dissolved in seawater.

von Holt and von Holt (1968) observed that various marine coelenterates, *Zoanthus*, *Condylactis*, *Stoichactis* and *Scolymia*, all of which contain intercellular zooxanthellae, readily and quickly take up carbonate when exposed to light. After a 3-hour experiment, a quarter to a third of the total photosynthate is found in the animal cells. Little information was gained on the method of transport from the algae to the animal, but presumably the substance transported was carbohydrate. It is possible that the photosynthetically-fixed carbon is passed directly to the animal or it may be excreted into the open water and subsequently readsorbed.

Okaichi (1967) determined dissolved carbohydrates in seawater in the vicinity of Seto Inland Sea by the phenol sulfuric acid method, both on ordinary days and when red tides polluted the waters. The ordinary level of carbohydrate appears to have been about 0.5 mg/l, but during red tides the concentration rose to 1.2–6.8 mg/l. Though the carbohydrate was calculated as glucose, it is understood that other substances interfere. Wangersky and Guillard (1960), for example, found that media from a culture of *Amphidinium carteri* gives a characteristic blue reaction with N-ethylcarbazole. Dialysis removed a substance which caused the reaction. This material creates a fishy odor in old cultures and appears to be alkaline. They believe that this organism liberates either acetylcholine or some substance similar to it. The substance is clearly not a carbohydrate in the customary sense.

Certain carbohydrate acids have also been reported in seawaters. Creac'h (1955a, 1955b) demonstrated the presence of citric and malic acid in Atlantic coastal waters. The water inside the shell of the oyster, *Gryphaea angulata*, contained 6.77 to 12.7 mg/l citric acid. Citric acid tends to be photoöxidized in sunlight to give acetoin, formic acid, acetone and acetaldehyde. Koyama and

Thompson (1959) evaporated 8 liters of seawater to 2 liters, then extracted the concentrate with ether for 4 weeks to recover the non-volatile organic acids at pH 3. They identified malic, lactic and glycolic acids (Koyama and Thompson 1964a) in the waters of the Northeastern Pacific. Barnes and Finlayson (1963) showed the presence of lactic acid in seawater. Their analytical method depends upon conversion of lactic acid into acetaldehyde in strong sulfuric acid, then condensing the acetaldehyde with p-hydroxydiphenyl to give a purple color.

Carbohydrates may have important metal ion-binding properties in the life processes of the sea. Korringa (1952) discussed the mucus of oysters as the agent by which ions are collected and transported to the tissues. Aluminum, copper, iron, zinc, mercury and manganese are accumulated by mucus sheets. Gripenberg (1966) examined the effect of mannitol, a common seaweed metabolite, on boric acid in seawater. It is well known that hydroxy-acids such as citric, malic and lactic acids tend to bind calcium and other elements in complex ions. Debourge and Creac'h (1964) demonstrated that a large number of complexes are made between carbohydrates; *e.g.*, mannitol, inositol, mucic acid, tartaric acid, pyruvic acid, oxalacetic acid, β-hydroxybutyric acid, etc., with tyrosine and hydroxyproline. These coordination complexes bind sodium ions and possibly other ions. The novel amino acid from diatom cell walls, 2,3-*cis*-3,4-*trans*-3,4-dihydroxyproline, would be expected to have powerful metallic ion-binding properties (Nakajima and Volcani 1969). Certain high molecular weight substances, possibly carbohydrates, influence the solubility of calcium carbonate (Park and Hood 1959). A part of the calcium bound by the unknown substance cannot be dialyzed. Rona *et al.* (1962) coprecipitated seawater organics with $Fe(OH)_3$ then extracted them with diethyldithiocarbamate to identify organic complexes of zinc and manganese. A substantial part of the manganese and zinc exist in non-dialyzable and nonextractable form. Matsudaira (1951) showed that citric acid and several other carbohydrates had a powerful inhibitory action on hydrogen peroxide decomposition by seawater. About 4–5 mg/l of citric acid is required to stop catalytic decomposition. Shapiro (1965) isolated some aliphatic, polyhydroxy carboxylic acids, MW 200–400, which seem to be capable of peptizing and holding large quantities of metallic ions. These may be the same as the humic substances fractionated by Gjessing (1966). He passed some humus-rich Norwegian seawaters through Sephadex gel columns to remove successively the different molecular weight ranges. The degree of association of colored molecules with iron appears to be pH dependent (Ghassemi and Christman 1968). The color is at a minimum at pH 7–8.

In contrast to these findings, Duursma and Sevenhuysen (1966) were unable to prove that metal ions were bound to organic ligands in the sea. They tested the effect of arabinose, isoleucine and other substances on the solubility of ferric hydroxide, zinc carbonate, nickel hydroxide and cobalt hydroxide. They could not demonstrate any appreciable difference in the solubilities of the metal ions in the presence of these organic substances. Further, the natural organic substances did not have any clear effects when added to the samples, even at high concentration levels. In view of the work of Traube and Kuhbier (1932) on olation complexes of iron with alkaline earth metal ions in solutions containing polyols, this work

might well be reexamined. Traube and Härting (1933) showed that very high pH is required to effect precipitation of the metal ions from such complexes.

Organic Nitrogen Compounds in Seawater

Ammonia can be estimated in seawater by distilling a portion, then applying the Nessler test (Konnov 1962). Gillbricht (1961) oxidized the NH_3 with sodium hypobromite and allowed the excess hypobromite to react with Bordeaux Red. The color difference between the sample and a blank is a measure of the NH_3 content. Many natural organic nitrogen compounds interfere, hence it is preferable to conduct the test after distilling the ammonia from the solution. Several similar colorimetric methods have also been suggested (Richards and Kletsch 1964; Newell and Dal Pont 1964; Roskam and de Langen 1964; Konnov 1965).

Hydroxylamine occurs in anoxic waters. It can be determined at the 0.015 to 3 μg-atom N/l level using sulfanilic acid colorimetrically (Fiadeiro, Solorzano and Strickland 1967). It is possible that many of the earlier reports of "ammonia" in seawaters may be in part attributable to the presence of hydroxylamine. Urea can be determined by reaction with biacetyl monoxime, semicarbazide and manganese chloride in a sodium dihydrogen phosphate buffer solution (Newell, Morgan and Cundy 1967). This test has no interference from NH_3, leucine, tyrosine, cystine, histidine, arginine, lysine or taurine. Concentrations of 1–20 μg/l may be estimated with fair precision by the procedure.

Chau and Riley (1966) reviewed published methods for concentrating amino acids from seawaters. None of the methods gave better than 50% recovery when the amino acids were present in concentrations below about 10 μg/l. They worked out a method which allowed recoveries higher than 90%. Thin-layer chromatography of the concentrate gave semi-quantitative values for the concentration of each of 19 added amino acids. Leucine and isoleucine could not be resolved, and cystine and tryptophane appear to be lost in the process. Nevertheless, 15 of the 19 amino acids could be recovered at concentrations of only 1 μg/l. Several of these amino acids were found in the waters from the Irish Sea.

Hobbie, Crawford and Webb (1968) determined the amino acid flux by estimating the individual concentrations of amino acids before and after incubation under given conditions (Table 1).

Siegel and Degens (1966) developed a ligand exchange chromatographic method for separation of amino acids from seawaters. Copper-loaded Chelex-100 resin (made by Bio-Rad Laboratories, Richmond, California) gave 100% recovery of all the introduced amino acids, except cystine, which was converted to cysteic acid and recovered in that form. They reported values for the amino acid composition of particulate matter, total dissolved and free amino acids present in seawater from Buzzards Bay. They conclude the bulk of amino acids dissolved in the sea are present in combined form rather than free. Other than peptides, it is plausible that these amino acids are coordinated in charge-transfer complexes with phenol-quinone type molecules. The presence of two hydroxybenzoic acids in the hydrolyzed seawater samples tends to support this hypothesis. Park *et al.* (1962) investigated the combined amino acid composition of seawater and Tatsu-

TABLE 1

Composition and concentration of amino acids as determined by Hobbie, Crawford and Webb (1968)

Amino Acid	Concentration μg/l
Glycine	16.85
Methionine	1.31
Serine	4.91
Alanine	1.49
Aspartic acid	1.92
Valine	0.82
Threonine	1.50
Leucine	0.63
Glutamic acid	1.00
Isoleucine	0.57
Lysine	0.95
Tyrosine	0.75
Proline	0.58
Arginine	0.55
Histidine	0.95
Phenylalanine	0.18
Ornithine	3.17

moto *et al.* (1961) estimated the amino acids in surface waters by the iron coprecipitation method.

Litchfield and Hood (1965) developed an assay for biotin using mutants of *Serratia marinorubra* and applied it to the determination in waters of the Gulf of Mexico and adjacent bays. Few of the samples contained biotin. Some of the samples contained a toxic principle which rendered the test useless. Aubert (1967) has reported that phytoplankton possess a thermolabile factor with antibiotic activity against *Escherichia coli* but not against fecal streptococci. Litchfield and Hood (1965) found a sample from Aransas Pass contained 1.6 μμg/ml of biotin. Off Port Aransas, a sample collected at 20 m contained 4.4 μμg/ml and one at 60 m depth gave 4.6 μμg/ml. During the spring and early summer, the quantity of biotin apparently increases and can be measured more readily. Mutants of the same organism were developed by Litchfield and Hood (1966) for determination of adenine, uracil and threonine. No threonine was found in any of the samples taken from the Gulf of Mexico. Uracil was detected only in water collected near the Mississippi River outflow. Adenine was found present in 7 different samples located at 4 different stations. Many of the samples contained toxic factors, though this factor was absent from the spring and early summer samples.

Degens, Reuter and Shaw (1964) found urea in the surface water off the coast of California, along with an unexpectedly uniform distribution of dissolved free amino acids. Combined amino acids decrease markedly within the first few hundred meters of depth and remain constant thereafter.

Fogg (1952) reviewed the extracellular nitrogenous products generated by several blue-green algae in young cultures. The chief constituent seems to be a polypeptide, along with smaller amounts of an amide.

Hellebust and Guillard (1967) demonstrated that the marine diatom, *Melosira nummuloides*, takes up amino acids from seawater at initial rates similar in magnitude to the rate of photosynthesis. Carbohydrates, polyols or organic acids are not similarly assimilated. This diatom is peculiar in its demand for certain types of nitrogen suppliers. Arginine is the favored nitrogen source, but glutamine, asparagine, proline and glutamic acid are also accessible to the organism. All other amino acids tested were unsatisfactory as nitrogen sources. The rate of amino acid assimilation declines rapidly with time, possibly owing to the build up of amino acid pools within the cells. Guillard (1963) surveyed the growth of several marine diatoms including some of the more common types of coccoliths. He examined their growth in the presence of such nitrogen suppliers as nitrate, nitrite, ammonia, urea, uric acid, glycine, glutamate and glutamine. Glutamate and glutamine were utilized by several organisms, but he found no evidence that organic nitrogeneous compounds in seawater was actually used by any algae. Provasoli and McLaughlin (1963) showed that arginine and asparagine could be utilized by three different *Gyrodinium* and two *Amphidinium*, glycine by two *Amphidinium*, *G. californicum* and *G. splendens*, glutamate and alanine by two *Amphidinium* and *G. splendens*. Though an attempt was made to find a common amino acid useful to all these dinoflagellates, it seems clear that some consume one kind and others another from the pool available in the dissolved amino acids of seawater. Evidently, the algae compete for certain organic materials and do not compete for others.

Stephens and Schinske (1961) noted that 35 species of ten phyla of marine animals could assimilate dissolved free amino acids from seawater. Webb and Johannes (1967) report that zooplankton release glycine, taurine and alanine along with other protein amino acids into seawater. The rate of release appears to be related to the temperature.

Since nitrogen compounds are known to be better chelants for metallic ions because of the superior donor properties of the amine nitrogen, amino acids might be expected to be particularly useful in chelating trace metal constituents needed by plants and animals in the sea. Johnston (1964) showed chelated trace elements were more effective than vitamins in bringing about an improvement in seawaters for many organisms. The supply of chelating agents is frequently the most crucial aspect of phytoplankton nutrition. Thiamine, biotin and the various B_{12} vitamins may themselves be chelants of unique importance to the nutrition of marine organisms.

Copper sometimes occurs in seawaters as an ultrafilterable, but nondialyzable, form (Slowey, Jeffrey and Hood 1967). They suggest it may be associated with a phospholipid, amino lipid or porphyrin fraction. Williams (1969b) confirmed these observations on several samples of Pacific Ocean water. There seems to be no correlation between the carbon content and copper in either organic or inorganic form.

Albert (1950, 1951, 1953) has compared the avidity for trace metals of naturally occurring substances, particularly amino acids, pteridines, riboflavin and purines. Bidentate amino acids exhibited preference for copper over nickel, which was greater than zinc, then cobalt, cadmium, iron, manganese and finally mag-

nesium. Approximately the same sequence, was followed for tridentate ligands; *e.g.*, tyrosine, lysine, ornithine, arginine and aspartic acid. Unlike the amino acids, complexes of the pteridines have some specificity towards trace metallic ions. Siegel (1966) examined the equilibrium binding of zinc-glycine complexes towards ion exchange resins and clays. He found that the zinc-glycine complex ion is almost as active as the Zn^{++} itself. He comments, "The quantities of trace metals in the sea and the distribution of these trace metals between the solution and the various solid phases such as sediments and detritus, must to some degree, be related to the presence or absence of various complexing agents in sea-water." Carbamino-carboxylic acids can be used by the phytoplankter, *Nitzchia closterium*, as an auxiliary source of carbon (Smith, Tatsumoto and Hood 1959). It has unquestioned chelant activity, which may conceivably be related to its uptake.

Menzel and Spaeth (1962) determined the occurrence of vitamin B_{12} in the Sargasso Sea. Droop (1957) has discussed the nutrition of marine phytoplankton with especial consideration given to the effects of vitamin B_{12}. Pintner and Provasoli (1958) found B_{12} is essential for growth and proliferation of the red-pigmented blue-green alga, *Phormidium persicinum*. This alga is capable of using asparagine as a source of nitrogen. Starr and Sanders (1959) reviewed the ecological aspects of the various vitamin B_{12}-active substances. Growth of the algal flagellate, *Gymnodinium breve*, is stimulated by vitamin B_{12} family of cobalt complexes. The vitamin B_{12} required for human metabolism, cyanocobalamin, is distinguished from the other vitamin B_{12} family by the nucleotide containing the base, 5,6-dimethylbenzimidazole. More than a dozen analogs with various structural modifications have been identified. It appears that few bacteria can produce all of the family and that comparatively few other organisms can substitute one member of the family for another. It is possible that the base for the nucleotide can sometimes be supplied by other sources than the preformed vitamin, thus riboflavin can supply the need for B_{12} by furnishing the base, 5,6-dimethylbenzimidazole, to bacteria which synthesize cyanocobalamin.

Thiamine occurs in seawater (Gold 1968) and is protected by the presence of sodium glutamate. Presumably copper ions catalyze its decomposition. Natarajan and Dugdale (1966) employed the marine yeast, *Cryptococcus albidus*, for bioassay of thiamine in seawater. They made a brief survey of the thiamine content of the North Pacific Ocean by use of this bioassay. Thiamine occurs in a range from the undetectable; *i.e.*, below 10 pg/ml up to 490 ng/l. It is generally higher in coastal regions and lower in open waters. The concentration decreased with depth down to about 50 to 75 m, below which the values diminish below the limit of detection. As a rule, thiamine followed the temperature distribution, with higher concentrations in the thermally stratified layer above the thermocline.

Budd and Spencer (1968) have found six species of bacteria and a marine yeast which are able to use alkylated amines as the sole nitrogen source. The constitutative ability involves dealkylation of such amines as methylamine, dimethylamine, trimethylamine and its oxide, ethylamine, diethylamine and triethylamine.

Hydrocarbons, Lipids and Fatty Acids from Seawater

Slicks have been known on the sea surface for a long time. Shuleikin (1935),

Schmidt (1936), Dietz and Lafond (1950) and Ewing (1950) agreed that monomolecular films, probably made by phytoplankton, cause the characteristic dampening of capillary waves called slicks. Some seawater samples from slicks are said to contain up to 27% organic matter. Paterson and Spillane (1968) noted the relation of kelp beds and slicks on the sea. Jarvis (1969) employed surface tension and surface potential measurements to detect adsorption of surface-active substances at the sea-air interface. It sometimes took several hours to secure significant decreases in surface tension; *i.e.*, attain measurable adsorption at the air interface. When a stream of air bubbles is sparged through the water, however, the surface is enriched quickly with surface-active materials. The slick-forming material can be isolated from the sea surface by coprecipitation with ferric hydroxide and then extracted with chloroform. The chloroform is removed under reduced pressure and the material applied to artificial seawater (Jarvis 1967). Ultraviolet photoöxidized natural seawater may be used instead of artificial seawater. It has none of the adventitious impurities characteristic of artificial seawater (Hamilton and Carlucci 1966).

Monomolecular films on seawater change the surface potential, tension and viscosity. Garrett and Bultman (1963) applied various pure, monolayer-forming substances to the surface of artificial seawater and observed the capillary-wave dampening. Substances such as oleic acid, oleyl alcohol, phenylstearic acid, cetyl alcohol, triolein and crystalline ovomucoid were tested. Several of these compounds act to stabilize air bubbles at the surface (Garrett 1967a). Curiously, the presence of a sea slick (*i.e.*, a compressed monomolecular layer) tends to reduce bubble stability and act as an antifoaming agent. This occurs even though the underlying waters may be rich in bubble-stabilizing substances. Samples of sea slick materials have been skimmed off the ocean surfaces in the Atlantic and Pacific Oceans, the Gulf of Lower California, the Gulf of Mexico and the Bay of Panama (Garrett 1967b). Most of the organic constituents seem to be fatty esters, free fatty acids, fatty alcohols and hydrocarbons. The distribution of fatty acids and their alcohols depends on various meteorological and oceanographic conditions for any given location at sea. There is a competition for the sites at the air-sea interface which results in the more active material replacing the less active material. This competition tends to fractionate the fatty acids and alcohols, which are the more surface-active, from the esters, hydrocarbons and similarly less-active substances. Hashimoto and Yasumoto (1960) observed that starfishes in aquaria produce foaming in the water when they die. The substance liberated is a saponin. On hydrolysis, the carbohydrate portion furnishes glucose, xylose, glucomethylose and 3-O-methylglucose. The identity of the aglycone is unknown.

Shabarova (1956) grew a number of marine algae under laboratory conditions and showed they elaborate several fatty acids; *e.g.*, formic, acetic and butyric acids. When the plants were permitted to decompose, methane was obtained. No ethane, propane or butane was observed with the organisms tested. Baker (1966) suggests that gas chromatography offers some advantages for the determination of volatile fatty acids in aqueous solutions. At the current state of development, this procedure is unsuitable for determining the low level of volatile fatty acids present in seawater.

Williams (1969a) recovered the organic acids of Pacific Ocean water by (a)

solvent extraction with carbon disulfide or chloroform and (b) coprecipitation with ferric hydroxide. Residues from CS_2 extraction or chloroform extraction were esterified with methanol and the methyl esters separated gas chromatographically. The even-carbon fatty acids from C_{12} through C_{22} were represented in all the samples. Fatty acids with C_{15}, C_{17} and C_{19} compositions apparently are present in some of the water samples as well. Certain regions have particularly high concentrations, 10–15 μg/l, of C_{17} acid. The efficiency of recovery was ascertained by using C^{14}-tagged palmitic acid. Samples of seawater from various depths were "spiked" with a given quantity of palmitic acid-1-^{14}C and the mixture subjected to analysis. Recoveries from 80.5 to 98.1% were obtained. Koyama and Thompson (1959) extracted seawater with chloroform or with ethyl ether and identified free acetic acid, formic acid, lactic acid and glycolic acid. Chloroform is somewhat more convenient to handle in continuous extraction systems. They (1964b) used partition chromatography to avoid some of the problems associated with simultaneous extraction of boric acid. The crude extract was passed through silica gel columns then eluted with a mixture of chloroform containing increasing amounts of *tert*-butyl alcohol. Boric acid, known to be present in seawater, was extracted by ether and on elution tended to depress the peaks of the organic acids, especially the hydroxy-acids. The extraction and partition chromatographic method was calibrated using acetic, formic, lactic, glycolic, malic and citric acids. The latter two acids, reported by Creac'h (1955), could not be detected. Koyama and Thompson (1959) gave values for the ranges and averages of these organic acids in Northeast Pacific seawaters (Table 2).

Slowey, Jeffrey and Hood (1962) found that ethyl acetate is a very efficient solvent for most of the kinds of lipids expected to be present in seawater. Sterols and sterol esters are somewhat more readily recovered with petroleum ether. By use of this technique, they identified capric, lauric, myristic, palmitic and stearic acids, and C_{14}, C_{16}, C_{18} and other unsaturated acids. Because the solvent is removed by distillation in a current of nitrogen, low molecular weight acids are generally lost. By a combination of solvent extraction, silicic acid column, thin-layer and gas chromatography, followed by infrared spectrophotometry, Jeffrey *et al.* (1964) showed that water from Redfish Bay, near Aransas Pass, Texas, contains a very complex mixture of lipid-soluble materials. They extracted 15-gallon samples of seawater with ethyl acetate, evaporated the solvent *in vacuo* and took up the sample of lipids in petroleum ether. A silicic acid column was carefully

TABLE 2

Concentrations (mg/l of seawater) of organic acids in northeast Pacific waters (Koyama and Thompson 1959)

	Surface		Inshore surface		Inshore bottom	
	Range	Average	Range	Average	Range	Average
Acetic Acid	0.09–0.82	0.37	0.72–2.83	1.83	0.07–0.86	0.39
Formic Acid	0.03–0.38	0.18	0.06–1.03	0.46	0.05–0.34	0.15
Lactic Acid	0.03–0.08	0.06	0.02–0.11	0.07	0.00–0.13	0.07
Glycolic Acid	0.06–1.04	0.51	1.32–1.40	1.36	0.24–1.07	0.66

prepared by washing with ether, acetone-ether mixture, ether again and finally rinsing several times with double-distilled petroleum ether, to assure that no traces of fat-soluble material were introduced to the sample. When the petroleum ether solution of the seawater lipids was eluted from the column, the first fraction contained chiefly paraffinic hydrocarbons, the second unsaturated hydrocarbons, the third sterol esters, the fourth triglycerides, free fatty acids and alcohols, the fifth vitamin A alcohol, vitamin D_3 and unesterified steroids, the sixth contained diglycerides and tributyrin, the seventh contained monoglycerides, vitamin A acetate and lithocholic acid and the eighth contained chimyl alcohol, phospholipids and monoacetin. When the first fraction was collected and subjected to gas chromatography, C_{10}, C_{12}, C_{14}, C_{16} and C_{18} hydrocarbons were recognized. The fatty acids of all fractions indicated both odd and even carbon acids from C_{10} to C_{22} are present in varying amounts. Many of the fatty acids were unsaturated. Fraction three apparently contains nonadecanoic acid in a quantity nearly equal to that of palmitic acid and greater than the quantity of oleic acid.

Jeffrey (1966) supplied values for the concentration of lipids in seawaters from various locations (Table 3) and the percentage composition of the various fractions, as obtained by Jeffrey *et al.* (1964), are shown in Table 4. The higher fractions were eluted with greater concentrations of ethyl ether in the petroleum ether developer solution. Fraction VII was eluted with pure anhydrous ethyl ether and the final fraction VIII was washed out with absolute methanol.

Williams (1965) estimated the dissolved fatty acids from various regions of the Pacific as methyl esters. He found much lower values than others have reported. As might be anticipated, the saturated C_{14}, C_{16} and C_{18} acids were present in much higher concentration than unsaturated acids. He suggests that "From all the carbon values reported for the Pacific . . . , it is evident that the total lipid values of Jeffrey *et al.* (1964) (8.65–13.8 mg/l of lipid or 3.45–5.52 mg C/l, taking carbon as 40% of the isolated lipid material) is greater than the total dissolved and can be accounted for only by some unique biological situation." He comments

TABLE 3

Concentrations of lipids in seawaters from various locations as measured by Jeffrey (1966)

Location	Depth (meters)	Date	Concentration mg/l
Galveston Texas Pier	1	June, 1963	9.10
Redfish Bay, Texas	1	May, 1963	7.70
Corpus Christi Bay, Texas	1	December, 1964	2.95
Laguna Madre, Texas	1	December, 1964	0.95
Port Aransas, Texas	1	December, 1964	1.29
Sigsbee Deep, Gulf of Mexico	10	August, 1963	2.40
	900		
	3400		
Peruvian Trench, off the coast of Chile	10	July, 1962	1.60
	1500		
	4000		
English Channel, offshore Plymouth	1	November, 1963	0.60
North Sea	1	April, 1944	0.40

TABLE 4

Percentage composition of various fractions of an extract of a 15-gallon sample taken in surface offshore water at 27° 17′N, 96° 56′W near Port Aransas, Texas (Jeffrey *et al.* 1964)

Carbon No.	Percent Fraction No. III	IVA	IVB	VI	VII	VIII
<10:0	...	...	1.80	5.10	2.21	36.74
10:0	...	0.09	0.49	0.71	0.71	...
11:0	...	...	...	1.23	0.45	...
12:0	...	0.32	3.71	6.76	5.25	4.79
13:0	3.95	...	0.38	0.78	0.89	...
14:0	4.36	3.91	2.38	1.69	1.61	8.62
14:1	...	0.26	...	...	...	0.76
15:0	1.65	3.13	0.42	...	...	0.96
16:0	21.48	17.14	5.41	8.51	4.03	11.51
16:1	7.67	0.16	1.42	...	...	...
16:2	3.38	0.32	...	...	...	...
16:3	..	4.52	0.66	...	...	...
17:0	4.95	.	0.14	...	2.32	...
18:0	5.95	10.00	7.09	2.12	8.92	7.18
18:1	14.06	52.68	26.60	11.36	10.87	7.82
18:3	3.95	1.42	1.81	2.60	...	3.96
18:4	...	3.67	0.73	...	...	...
19:0	21.14	...	...	...	2.88	...
20:0	4.10	1.06	...	...	...	...
20:2	...	0.98	15.20	27.18	...	...
20:3	...	0.16	0.36	2.82	...	3.20
20:4	3.30	0.16	1.82	...	6.0	...
20:5	...	...	8.24	3.37	17.86	4.83
22:4	...	..	...	3.14	...	...
22:5	...	...	...	...	...	9.50
22:6	...	...	...	2.70	...	...
<20 unidentified	...	...	21.40	19.94	36.02	...

further that "The origin of fatty acids in sea water is not evident from a comparison of their individual distribution patterns compared with those found in phytoplankton, zooplankton or in higher trophic forms. The absence of the C_{20} and C_{22} polyunsaturated acids, characteristic of marine zooplankton and fish, may be due to oxidation insufficient sensitivity of the method used."

Matthews and Smith (1968) extracted the sterols from seawaters off Galveston using hexane, pooled the samples and chromatographed them on silica gel to obtain concentrates suitable for gas chromatography. In this way, they established the presence of cholesterol, stigmasterol and β-sitosterol. They discovered in the course of their study that Negal plastic bottles donate small quantities of dimethyl, dibutyl and diisooctyl phthalates to the water samples, hence it is desirable to use glass bottles for the collection of seawater samples. Certain of the samples contained a substance which may be campesterol. None of the higher methylated sterols (*e.g.*, lanosterol) or unsaturated sterols (*e.g.*, ergosterol *or* 7-dehydrocholesterol) were detected. Surprisingly, they were unable to demonstrate the presence

of oxidized sterol derivatives in seawater. Neither microbial nor photoöxidation products could be detected. This is all the more remarkable in view of the known microbiological reactions of sterol compounds (Kogan 1962).

Mallet and Heros (1961) first called attention to pollution of the sea by carcinogenic hydrocarbons, particularly fused-ring aromatics such as 3,4-benzopyrene. Chemical, chromatographic and spectrophotometric methods indicate the presence of benzo-3, 4-pyrene in both animals and plants; *e.g.*, molluscs, echinoderms, crustaceans and fish (Mallet 1961). Traces of benzo-3, 4-pyrene and similar hydrocarbons in the seawater can thus be accumulated by organisms. Lalou, Mallet and Heros (1962) identified these substances by fluorescent spectroscopy in the muds of the bay of Villefranche-sur-mer. Sediments from the Arctic Ocean also contained fused-ring aromatics of the same type (Mallet, Perdriau and Perdriau 1963), seemingly indicating there is general pollution in the northern hemisphere. Plankton from Villefranche contained up to 7.5 γ of benzopyrene/100 gm (Mallet and Sardou 1964). It is assumed that the pollution comes chiefly from the atmosphere and that petroleum refineries, coke ovens, furnaces and power stations are the chief cause. Plankton in the Rance estuary contain small amounts of these hydrocarbons (Mallet and Lami 1964). Surprisingly, Mallet and Schneider (1964) found 1.95 γ benzo-3, 4-pyrene/100 gm of limestone from 50 m depth in the Gouvieux mine and from other geological sources. Mallet (1965) reviewed this work and described the concentrations of benzopyrene in the bay of Villefranche at the Rhone Delta.

Scaccini Cicatelli (1965, 1966a, 1966b) found the fresh water annelid, *Tubifex*, would accumulate benzopyrene in fresh water aquaria and eliminated it only very slowly when placed in an unpolluted environment. The waters of the aquaria were polluted with 100 γ, 1 γ and 0.01 γ of benzopyrene per liter. The worms contained, after 6 days exposure, respectively 1,350γ/100 gm, 380 γ/100 gm and 22.4 γ/100 gm, while the control aquarium had worms with only 7.5 γ/100 gm of tissue. Benzopyrene is also accumulated by marine organisms and has been detected in numerous species in the upper and middle Adriatic (Piccinetti 1967). The hydrocarbon apparently accumulated without alteration by a wide variety of species, none of which metabolize or detoxicate it, and it is excreted very slowly if at all.

Niaussat, Ehrhardt and Ottenwalder (1968) found significant concentrations of benzopyrene in the lagoon water of Clipperton atoll. This discovery in a biologically-rich region largely free of industrial pollution tends to suggest the possibility of biosynthesis of this kind of fused-ring aromatic hydrocarbon. Since these substances are not readily metabolized, they accumulate through the food chains.

Blumer, Mullin and Thomas (1964) suggested that the hydrocarbon, pristane (2, 6, 10, 14-tetramethylpentadecane), occurs among the lipids of dissolved organic matter in seawater because of its partial solubility. Pristane occurs in *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus*, in quantities amounting to 1–3 % of the body fat. These krill serve as the food supply for whales and other large sea animals, hence the content of pristane in these oils (Blumer, Mullin and Thomas 1963). It may be possible to trace food chains by means of hydro-

carbons such as pristane or benzopyrene because of their comparatively insignificant metabolism.

Since petroleum is generally believed to be formed in anoxic environments, the organic composition of oxygen-deficient seawaters is especially interesting. Adams and Richard (1968) extracted the acidified waters from Nitinat Lake, adjacent to Vancouver Island, with petroleum ether and ethyl acetate. The residue from ethyl acetate extracts gave dark yellow-brown solids, while the petroleum ether residues were nearly white to a light yellow. There seems to be more organic matter in the anoxic water (1.8 to 1.9 mg/l) than in nearby oxygenated waters. The extracted residues correspond to approximately 40–55 % of the dissolved organic matter. The petroleum ether extracts evidently contain sterols or sterol esters, but the ethyl acetate residues were free of these substances. On the other hand, choline-type phospholipids are present in the ethyl acetate extracts but absent from the petroleum ether residues. Curiously, the anoxic waters, when extracted with ethyl acetate, showed the presence of a sulfhydryl compound of some kind. The precise nature of the mercaptan-like substance is not known.

The Black Sea is a large anoxic basin. Ushakov *et al.* (1966) extracted 50 l of filtered surface water with ethyl acetate, methylated the residue with diazomethane and chromatographed the esters on silica gel thin-layer plates. They obtained 5 fractions, amounting to 342 mg/m^3 of the seawater. Each of these fractions was examined by gas chromatography and all the saturated acids from C_{11} through C_{20} found, along with several unsaturated C_{15} to C_{17} acids, oleic and linoleic acids. Vityuk (1967) prepared a detailed survey of the fatty acid fractions from 200, 500 and 2000 m depth. He found that unsaturated acids constituted nearly half of the total fatty acids of these Black Sea waters.

Acrylic acid was identified by Sieburth (1960) as the antibiotic derived from an alga, *Phaeocystis*, which inhibits the intestinal flora of penguins. Its occurrence in seawater, however, has not yet been attested. Its precursor, dimethyl-β-propiothetin, occurs in a great many species of marine multi- and unicellular algae. Ackman, Tocher and McLachlan (1966) found many species of free-living algae liberated dimethyl-β-propiothetin when treated with cold, aqueous alkali. Degradation of dimethyl-β-propiothetin gives dimethyl sulfide and acrylic acid. Many filter-feeding molluscs concentrate this thetin, probably from the green algae, *Prasinophyceae*, of which it is characteristic (Ackman and Hingley 1968).

The lower fat-soluble substances are comparatively little known. Swinnerton and Linnenbom (1967) worked out a method for determining small amounts of dissolved gaseous hydrocarbons (*e.g.*, methane, ethane, propane, butane, isobutane, isopentane and n-pentane) in seawater. Hydrocarbons as high as n-octane can be determined by the same technique. The seawater sample, 700–900 ml, is placed in a specially-designed stripping chamber and the hydrocarbons and other gases removed in a current of helium. The hydrocarbon gases are concentrated in a series of cold traps and subjected to gas chromatography for separation. It is not possible by this technique to distinguish ethane from ethylene or propane from propylene, if present. Nothing is known of the total dissolved organic carbon in these samples. Specimens of seawaters from the Tongue of the Ocean in the

Bahamas and from Chesapeake Bay were analyzed by this procedure. It is noteworthy that the gas chromatography scans showed several unidentified peaks, some of them quite substantial, among the hydrocarbons.

CONCLUSION

Previous reviews of the organic composition of dissolved organic matter in seawaters (Vallentyne 1957; Provasoli 1958; Duursma 1960; Provasoli 1963; Kamatani and Matsudaira 1966; Koyama 1967) have particularly focused on the very low concentrations of dissolved carbonaceous substances in seawaters. Because of the improbability that such low concentrations would be consumed or used as a trophic base by marine organisms, various means for identifying how plants and animals might concentrate or collect these nutrients were examined. It was demonstrated that Millipore-filtered seawater could be sparged with air to produce particulate matter (Baylor, Sutcliffe and Hirschfeld 1962; Sutcliffe, Baylor and Menzel 1963; Riley 1963). These nonliving aggregates contain material useful for the growth and development of certain organisms (Baylor and Sutcliffe 1963). There is no doubt that these small particles have some importance in the life of the sea (Siegel and Burke 1965; Sheldon, Evelyn and Parsons 1967; Johannes 1967). The various experimental methods for producing these aggregates have been reviewed by Batoosingh, Riley and Keshwar (1969). They observe that various investigators have secured widely-varying results by seawater aeration. There is no evidence that bacteria are any more important to the formation of aggregates than any other matter. There appears to be a concentration beyond which there is no further formation of particulate matter. When the particles are harvested, they are replaced by new particles. How this occurs is not clear. No serious artefact could be detected in the work of the many investigators in this field of study.

Lucas (1936, 1938, 1944, 1947, 1949, 1955, 1958, 1961) has been arguing, from increasingly convincing data, for the past three decades that the external metabolites of marine organisms serve not only as growth factors but as foodstuffs for both plants and animals in the sea. The dissolved organic matter in seawaters, even at the unlikely concentration levels known, supplies this need. The naive hypothesis that land plants live exclusively upon mineral matter has long since been discredited. In effect, Lucas has now demonstrated that marine plants require certain organic nutrients as well. This review seems to support still further the idea that both marine plants and animals have a fundamental ecological relationship with the dissolved organic matter in the sea. In Boileau's still celebrated words, "*le vrai peut quelque fois n'etre pas vraisemblable*"; *i.e.*, the truth may sometimes be not probable.

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